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# **Planning Photography of Microfossils**

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PHOTOMICROGRAPHY using an optical system is a very specialized kind of photography. It involves many problems not encountered in the photography of large specimens with an optical camera or microscopic specimens with a scanning electron microscope. Within the near future, however, SEM facilities are likely to be available to only a fortunate few micropaleontologists, and the cost of producing large numbers of photographs will be prohibitive for most budgets. Commercially designed and produced optical photomicrography systems are also expensive, and most of them include features unnecessary for the particular usage intended. Hence, micropaleontologists seem destined for some time to planning their own system, utilizing insofar as possible whatever equipment may be already present in their organization or unit.

The following data and photographic "principles" are presented for use in planning and operating a "single-lens" (usually a coordinated set of lenses cemented together) camera. A few suggestions on films and printing are added for consideration, although success or failure in photomicrography centers on the design of the camera.

In "Photographic Fundamentals" (1961, Course 2320, Extension Course Institute, Air University, Gunther Air Force Base, Alabama), photography is described (p. 57) as

... a process made up of many miracles .... the miracle of optics -- how sharp, detailed, brilliant images can be formed by lenses and projected on a sheet of film .... the miracle of film, and the latent image that is formed on it .... the miracles of chemistry, by which the latent image becomes a visible one through the action of a developer, and then becomes permanent through the action of a fixing bath. This is a concise list of the stages through which each photograph must proceed. Thoughtful and careful planning is required so that each stage is adjusted and integrated with the others.

#### OPTICS

In the optical considerations -- the transfer of light from the subject to the film with acceptable clarity -- many factors are interrelated. The lighting of the subject determines the intensity and contrast of the image, as well as the portraval of the essential characteristics of the subject. The size of the subject and its magnification on film determine the size of the film and the degree of enlargement in printing. The focal length of the lens sets the size of the camera and its support. The optical design and accuracy of the lens and the diameter of the opening produce the depth of field in the final print. If all factors are "perfect" we have an accurate, exciting, and even dramatic interpretation of the subject; if all factors are planned and executed with moderate care, we have an acceptable picture; but if any one factor is wrong, we have nothing for our efforts except the experience in what not to do.

Light. -- Let us begin the discussion with light, since light is the essential denominator in all phases of photography. We are by necessity involved with the nature, properties, quality, and behavior of light from the time it bounces off the subject, makes its way through the lens, and penetrates the film emulsion, until we can "see" the final result as a series of light waves radiating from the print.

Light has dual sets of characteristics, both taking part in photography. This visible form of energy can act as a radial wave action, as in the waves emanating in all directions from the subjects we see (like waves spreading outward in a pool from the point where a pebble is dropped), or it can act as a quantum, as in the exposure of film during which an amount of light passes through the lens opening during the interval the shutter is open (like the quantity of water passing through the spout during the time the spigot is opened).

Sunlight contains waves or radiations of all colors in the visible band or spectrum, as well as those longer than the red end (infrared) and those shorter than the violet end (ultraviolet). That is why it is ideal illumination: whatever the wavelengths to which the film is sensitive, they are present in sunlight.

The electric bulb, fluorescent unit, flash, or arc light used for artificial illumination has serious gaps in the spectrum of its light. The human eye is so marvelously accommodating to the owner that it is really a poor judge of the colors (or even the accurate intensity) of such light; the emulsion on the film is much more discriminating. Before selecting an illuminator the photographer should check its registration on the film with the optical system he plans to use. Filters should be added only if they improve the image on the film.

REFLECTION. When light waves strike the surface of the subject, they may be *reflected* or thrown back. If the surface is smooth and polished (like a mirror), the reflected light is thrown back at the same angle as the incident or incoming light, and the reflection is said to be *specular*. But if the surface is rough or irregular, the light is reflected in more than one direction and in more than one plane. Such reflected light is *diffused*.

As we see a microfossil under the microscope, we mentally adjust the image to emphasize what we wish to "see." If a specular reflection tends to obscure some detail, we simply ignore it ("filter" it out mentally) or we turn the specimen so that the polished surface reflects in another direction. If the fossil is transparent, we focus our attention on the outermost surface. And we subtly distinguish to ourselves whether a dark spot is a stain on the surface, the shadow of an elevation, or a de-



Reflections from surfaces.

pression that is not lighted. The film is impersonal; it registers the colors and their intensities as the light energy passes through the lens during exposure, impartially and finally.

With rare and special exceptions, the paleontologist seeks to portray the *form* of the fossil and *not* its color or polish. Therefore, when he photographs, he wants the specimen to give off diffused light. To bring this about, he usually coats the specimen with a very thin layer of sublimated ammonium chloride. The particles are uniform in color (white) and make the surface "rough." As long as the coating is not thick enough to obscure any structures of the fossil, and its particles are not large enough to be distinguished in the final print (conveying a false impression of texture), the ammonium chloride is successful in bringing out the configuration of the specimen.

TRANSMISSION. Light passing through an object is *transmitted*. Objects such as a lens or window pane, through which objects are clearly visible, are *transparent*. Frosted glass, which scatters the transmitted light so that objects cannot be seen clearly, is *translucent*. A medium which will not transmit light at all is said to be *opaque*.

Transparency is a matter for consideration with the lens. In old lenses, the cement between the lens elements may become discolored with age or lose some of its transparency; in either case, the lens is seriously im-



Direction of refraction,

paired in transmitting light faithfully, and it should not be used in such condition.

ABSORPTION. When light falls on an object and is neither reflected or transmitted, it is If our plates are to have a black absorbed. background, absorption is a factor to be reckoned with. With large fossils, the specimen can be mounted on a small pellet of clay or wax to elevate it above the black-card background; then when the specimen is coated, the ammonium chloride settling on the background can be wiped off with a moistened brush. Exposed and developed photographic printing paper (even with a matte surface) reflects some light. Black illustration board absorbs light far better and its rough texture tends to diffuse any reflected rays. With microfossils, the specimen

cannot readily be mounted above the background. The blackness of the background must be added on the plates with "photographic black" showcard color (Prang #834 Black Tempera Color diluted slightly is a satisfactory and cheap substance). This involves carefully outlining each printed picture with the black solution, but the process seems to be unavoidable.

REFRACTION. Another important property of light is that it can be "bent" as it passes from one medium obliquely into a substance of different density. The bending of light is called *refraction*. As a light ray passes at an angle from air into glass (denser than air), it is bent toward the perpendicular to the air-glass surface; and as the ray emerges from the glass into air, it is bent away from the perpendicular.



Focal length.

By refraction, a lens gathers light rays from distant subjects and concentrates them on the film.

Let us consider light rays radiating from a point source and a camera directed toward that source. If we replace the lens with a pinhole, light can enter through it into the camera and strike the film. If the light source is far away, the ray reaching the film will be scarcely larger than the pinhole through which it entered the camera. But if the source is quite close to the camera, light rays will spread through the pinhole as a cone of illumination, and the point source of the light will be represented on the film by a circle. This will be referred to later as the *circle of confusion*.

Now consider two pinholes several millimeters apart. Rays from the distant point source will enter each hole and register as two spots on the film. Yet if we center a lens behind the two pinholes, it will be possible to refract the two rays so that they coincide at a spot on the film. If the lens is formed correctly, the rays from the point source to every site on the lens surface can be refracted in such a way that they all converge on the film -which is said to be then at the *focal plane*. How closely all these images coincide determines the "sharpness" of focus and the value of the lens. If the light passes through more than one lens on its way to the film, any inaccuracies in the curvature of any of the lenses will affect the image at the focal plane. Hence, a single lens may yield better results than a series of lenses.

DIFFRACTION. As light strikes the edge of an opaque medium, it is scattered slightly. When lamplight passes by an opaque object, the shadow of the object has a "fuzzy" edge.



When a ray passes through a pinhole, all the perimeter of the hole is causing *diffraction* of the light. The only way to avoid any such scattering would be to make the pinhole in a metal plate of infinite thinness. Since this is impossible, the best way to reduce diffraction from the diaphragm of a lens is to use the thinnest metal suitable and to avoid very tiny openings. More will be said about this later under "Definition."

Lens. -- The lens is a device made of high quality and uniform glass so shaped that the light rays from any point of the subject, irrespective of where they fall upon the lens' surface, will be refracted to meet and form an image of the point. The point where these rays converge (actually, the very small area where they come together) is called the *focus*, and the plane on which the lens forms the sharpest image is known as the *focal plane*. Here the film is placed for exposure. The function of any lens is to project the image of a subject on the focal plane.

The lens is part of a system which incorporates a *diaphragm*, or adjustable opening, and a *shutter*, or valve. The diaphragm may be made larger or smaller to control the amount or intensity of the light being admitted to the focal plane. The shutter is opened for an interval of time to admit this light during exposure, and thereafter is closed to prevent additional and unwanted light from entering the camera. The longer the shutter is open, the more the quantity of light which passes through it. The film is "illuminated" only when the shutter is opened, and must be **k**ept in total darkness before and after the exposure until it is developed in the darkroom.

Focal length. -- The distance from the

center of the lens to the focal plane when the lens is focused on a point at infinity is the *focal length*. Three factors determine the focal length of a lens: curvature of the lens surfaces, the kind of glass used in its manufacture, and, in the case of "compound" lenses, the separation between the lenses or lens elements.

Rays from points closer than infinity strike the lens at increasingly greater angles and come to focus farther from the lens, behind the focal length. This change in the position of the focal plane results from the fact that the lens will refract rays by only a certain amount from the angle of incidence. As the subject is situated closer to the camera, the lens-to-film distance must be increased. And if the subject is very close to the lens, the camera must be lengthened by some kind of long bellows.

Image size. -- Provided the subject remains the same distance in front of the lens, the size of the image is directly proportional to the focal length of the lens. The longer the focal length, the larger the image size; if the focal length is twice as long, the image will be twice as high. In a 35-mm camera (with short focal length lens) the scene to be photographed is condensed onto the frame of 35-mm film; in a press camera (with a long focal length lens) the scene would be enlarged over the area of a 4 x 5-inch sheet of film.

The image size can also be increased by moving the lens closer to the subject. If it is impossible to move the camera close enough to the subject to register the details you desire, you can substitute a lens of longer focal length. Hence, a telephoto lens (with very long focal length) could be used on a 35-mm camera to photograph details of ornamentation on a distant cathedral. In photomicrography, the advantage of a longer focal length is the increased "working distance" between lens and subject at the same magnification -- more room in which to manipulate the subject and adjust the lighting.

Speed of the lens. -- The speed of a lens is determined by two factors: the diameter of the aperture through which light passes into the camera (controlling the amount of light admitted in a given interval of exposure) and the focal length of the lens (governing the distance the light must travel from the lens to the film). A



Relation of focal length to image size.

larger opening admits more light and is "faster"; the longer focal length decreases light intensity because the light must travel farther and is spread over a greater area of the film.

The speed is indicated by f-stops, also known as diaphragm settings or f/numbers, which are simply ratios of the focal length to the diameter of the opening. Thus a lens with focal length of 96 mm and a diameter of 12 mm has a speed of 96/12 = f/8. Similarly, a lens with a focal length half as long and the same diameter would have a speed of 48/12 = f/4.

The above speeds refer to lenses with the aperture wide open. However, lenses are equipped with an iris diaphragm which can be adjusted to control the light entering the camera. The standard f-stops on the diaphragm scale are: 1, 1.4, 2, 2.8, 4, 5.6, 8, 11, 16, 22, 32, 45, 64, 90. Scarcely any lens covers all this range of f-stops. Only special lenses have a maximum opening of f/1, since the diameter of the lens would have to equal its focal length. On the other hand, lenses used in scientific work may stop down to f/45.

Two important facts should be remembered about f-stops. First, they are fractions representing a ratio between focal length and diameter of the aperture. Thus, a setting of f/11represents an aperture that equals one-eleventh of the focal length, and therefore is smaller than an opening of f/8. The larger the f-stop number, the smaller the opening.

Second, as the lens is "stopped down" (made smaller) from one standard f-stop to the next, the light entering the camera is reduced by one-half. Obviously an opening of f/16 has half the diameter of an opening of f/8, and has only one-fourth the area through which light may pass. This relationship becomes clear as we square the f-stop numbers in the standard series above; the squares are: 1, 1.96, 4, 7.84, 16, 31.36, 64, 121, 256, 484, 1024, 2025, 4096, 8100. This series is not far from the ideal series formed by doubling: 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, 8192.

Some diaphragms are also calibrated for half stops at 3.5, 4.5, 6.3, and 9.5. Closing

down from one half stop to the next standard stop reduces the light intensity by about onefourth, but closing down from one half stop to the next half stop cuts the light intensity in half. This becomes clear as we square the half stops: 11.25, 20.25, 39.69, and 90.25. These squares are close to the idealized 12, 24, 48, and 96.

In actual practice, most cameras have stops that are accurately calibrated at f/4, f/8, or f/16, and the other stops are set exactly where the light is decreased or increased by one-quarter or one-half. Thus some of the labeled f-stops and the actual f-stop openings may differ slightly: Labeled - 1 1.4 2 2.8 3.5 4 4.5 5.6 6.3 Actual - 1 1.41 2 2.83 3.46 4 4.90 5.66 6.93 Labeled - 8 9.5 11 16 22 32 45 64

Actual - 8 9.80 11.3 16 22.6 32 45.3 64

<u>Circle of confusion.</u> -- Any subject being photographed is composed of an infinite number of points, each of which reflects light toward the camera. The lens creates an image of each of these points comprising the subject upon the film in such a way that their relative brightness will convey to the eye observing the print the essential features of the subject as it might have been seen directly at the time of the photographing. In short, the purpose of a camera lens is to produce a picture that clearly looks like the subject.

Not even the lens in the human eye can focus on subjects at all distances simultaneously. When the camera is focused at a certain distance, all the points in the subject at that distance will be registered on the film with reasonable accuracy, and when the film is developed they will be recorded as microscopic specks, closely corresponding in size to the grain of the film emulsion.



Circle of confusion.



Relation of subject distance to depth of field.

Points in the subject (or other subjects) which are farther away or closer to the camera will be projected onto the film as small circles of greater diameter than those at the focal point -- the diameter increasing as the point of convergence gets farther away from the focal point for which the lens is set. This small circle is the *circle of confusion*.

If the actual measurement of this circle of confusion is 0.25 mm or less in the print, it will be interpreted by the eye of the observer as a point when viewed from a distance of about 10 inches, and the photograph is considered "sharp." If it is larger than 0.25 mm, the eye distinguishes it as a circle instead of a point, and that part of the photograph appears blurred, "fuzzy," or "out of focus." This acceptable 0.25 mm limit on acceptable circle of confusion applies to the print; if the negative is to be enlarged by four times in printing, the circle of confusion in the emulsion of the film could only be 0.0625 mm. To place a practical value on negatives to be enlarged, the photographer should plan for a circle of confusion of about 0.1 mm.

When a lens is focused as accurately as possible, the circle of confusion will be the

smallest the lens can produce, and the spread will depend upon just how accurately the lens can bring all the rays of light from a point to converge on the focal plane. This circle is then called the *measurable circle of confusion* and is a fixed property of the lens. No lens is perfect. However, most lenses for scientific work have a measurable circle of confusion that approaches 0.07 mm.

Depending upon the design and care in manufacture, a lens may give a smaller or larger measurable circle of confusion for subjects at different distances from the lens. The only way to discover the properties of a particular lens is to try it for your subjects at the necessary distance to produce the desired magnification on the film.

Hyperfocal distance. -- This topic may at first seem out of place is a discussion of photomicrography, because it is commonly used in planning for distant subjects and particularly for subjects over a span of distance. Nevertheless, the hyperfocal distance is a factor that may be used in determining the depth of field for a given lens -- and depth of field is extremely critical in photomicrography.



Relation of aperture to depth of field.

When a lens is focused at infinity, the distance from the center of the lens to the nearest subject in acceptably sharp focus is the hyperfocal distance (HFD). This boundary between the sharp and the blurred portions of the image has a number of uses. When the camera is refocused on the HFD, the area of sharpness moves toward the camera to about half the HFD and at the same time, subjects far from the camera (said to be at "infinity") are still in acceptable focus. Focusing a lens on its hyperfocal distance gives the maximum depth of field for the f-stop being used. As shown in the discussion of formulas, the HFD is theoretically a function of the focal length of the lens, the f-stop, and the acceptable circle of confusion. Actually, of course, two lenses of the same focal length opened to the same f-stop may yield different HFD's; one of the lenses may be superior and produce sharp images of subjects from infinity to quite close to the camera, whereas the other lens may be inferior and scarcely give acceptable sharpness for subjects at infinity or any other distance.

The common use of HFD is in pre-planning a rapid series of action shots for sports events. For example, it is found that with the fast film being used in the camera, the light is strong enough to allow exposures of 1/1000th second at f/16. The lens has a focal length of 100 mm, and the circle of confusion is 0.1 mm; the HFD is calculated as 6250 mm. Hence, with the camera focused at 6.25 meters, all subjects will be in acceptable focus from 3.13 meters to infinity. Action shots can be taken in quick succession without changing any settings as long as the subject is over 10 feet away.

Depth of field. -- The distance from the nearest plane in acceptable focus to the farthest plane in acceptable focus for a particular setting of the lens and camera is called the *depth of field*. It is the measurable span of distance in front of the lens within which all subjects appear reasonably sharp on the focal plane. Since nearly all subjects have appreciable third dimension, or depth, the depth of field is extremely important in photomicrography. As discussed below, the depth of field can be calculated for lenses of average grade.

Several factors are concerned in this theoretical depth of field.

SUBJECT DISTANCE. As the subject is farther away (the distance from the lens to the subject is increased), the depth of field increases. The more distant the subject, the more nearly parallel are the light rays reaching the lens, and all these rays tend to converge in nearly the same plane. On the other hand, the closer the subject (the higher the magnification of the image), the greater the angle at which the light rays reach the lens, and the greater the cone of spread -- and therefore the less depth of field.

CIRCLE OF CONFUSION. The circle of confusion is directly related to the theoretical depth of field and to the actual depth of field. In the theoretical depth of field (the formula by which calculations can be made), the acceptable size of the circle of confusion on the film image is directly proportional to the (accepted) depth of field; hence, the more "tolerance" of a slightly blurred image, the greater the span which can be classified as within the depth of field.

In the actual depth of field (the concrete results produced by the particular lens), the subject in the vertical plane on which the lens is focused will have all points of its surface form images on the film composed of the smallest circles of confusion which the particular lens is capable of producing. Subjects in front or behind this vertical plane will register on the film in ever-increasing circles until the image is unacceptably "fuzzy." Therefore, the more nearly perfect the lens, the smaller will be its measurable circle of confusion and the greater will be its depth of field.

FOCAL LENGTH. As just mentioned, the depth of field depends to a significant degree upon the minimum size of the circle of confusion that the lens can produce -- its measurable circle of confusion. This is an inherent property of the lens and cannot be changed. A lens of average quality has a measurable circle of confusion that is approximately 1/1000 th of its focal length. Therefore, an average lens of short focal length will give a smaller circle of confusion and have more depth of field than a lens of longer focal length (at the same f-stop).

This is true for subjects at a fixed distance. For general outdoor photography, a lens of very short focal length in a minicamera has such depth of field that most manufacturers do not even provide such camera with a focusing device. These minicameras are "in focus" from a few feet to infinity. However, the image produced by such lenses is very small because of the very short focal length.

Photomicrography has very different goals than general outdoor photography. Instead of a slide or print of a distant scene, we are concerned with deriving a sharp image of given magnification on the film. As will be demonstrated in the formulas below, there is theoretically no difference in depths of field for subjects taken at the same magnification and at the same f-stop -- regardless of the focal length of the lens used.

DIAPHRAGM OPENING, *f*-STOP, OR APERTURE. The easiest and most frequently used method of controlling depth of field is by the size of the aperture. All other factors kept constant, the smaller the lens aperture, the greater the depth of field. Stopping down the diaphragm opening makes all the light rays entering the camera more nearly parallel, causing them to focus on approximately the same focal plane.

As a standard procedure in photomicrography, focus with the lens wide open on the *middle* of the desired depth of field. Then close down to the smallest opening practical for the particular lens, and check the actual depth at that position of focus. If the actual depth of field includes the closest spot of the subject that you wish to have in focus, then the camera is set for the exposure.

With all other factors equal, lenses with the same f-stop should give the same depth of field at the same magnification. Yet, as we shall see below under "Definition," all other factors are not quite equal, and the very short focal length lenses do not give as deep a field at very small openings because of diffraction.

Definition. -- The ability of a lens to reproduce fine detail is its *definition*, or *resolving power*. Two factors influence definition: the quality of the lens and the diffraction of light as it strikes the edge of the diaphragm. As stated before, the resolving power of any lens depends



Usable portion of the circle of illumination.

on its measurable circle of confusion, which is inherent in its manufacture. The design of any lens is never perfect, so the complete lens does not have precise focus of subjects positioned at various distances. Especially the edges of the lens, where refraction must be maximum, have geometric deficiencies or aberrations which greatly affect definition. Therefore, most lens units work better when stopped down from their maximum opening.

When the diaphragm is stopped down to a very small aperture, the diffraction of light glancing off the edges of the diaphragm causes loss in definition. This problem is acute in the smaller openings (f-stops) of short focal length lenses. For example, a lens of 16-mm focal length reaches a stop of f/45 when the opening is only 0.36 mm in diameter. This is too small for good resolution.

This, then, is the dilemma of the photomicrographer: to keep the camera in reasonable dimensions he needs a short focal length lens, and for depth of field he needs a small aperture; but the small aperture in a short focal length lens is so small that diffraction spoils the resolution and therewith the depth of field.

The best resolution for any lens is a compromise between closing down the aperture to take advantage of the fewer aberrations in the middle of the lens and opening the aperture to avoid diffraction on the **diaphragm** edges. Because of the diffraction problem with extremely short focal length lenses, they are to be avoided in photomicrography except for high magnifications which cannot be otherwise achieved.

Lens coverage. -- Light passing through a lens is projected to the focal plane in a circular shape called the *circle* of *illumination*. The intensity of light decreases outward from the center of this circle as the distance from the lens increases. Of course, the film cannot be "dished" into a concavity to make it parallel to the lens surface. To avoid serious problems of light intensity "fading out" toward the edges, only the center of the circle of illumination is used as the *lens coverage* on the film. To be sure that the central part covers the film, the rule is applied that the focal length of the lens should ordinarily be about the same size as the diagonal measurement of the film used. Thus, a camera using full-frame 35-mm film (about  $25 \times 35 \text{ mm}$ ) should have a lens of at least 43 mm focal length  $--(25 \times 25) + (35 \times 35) = 1850$  $= 43 \times 43$ . This rule must at times be violated in arriving at higher magnifications, but the problem of "dim edges" must be anticipated.

Equations. -- In the following sections, some of the equations dealing with optical properties of lenses and exposure are collected as a handy reference for the micropaleontologist and would-be photomicrographer. For each topic, an example or two is given and the generalized principles are stated. 

- ASA = film speed
  - b = distance from lens to film
  - B = distance from infinity position to film
  - c = acceptable circle of confusion
  - d = diameter of lens opening; the f-stop
  - e = relative exposure time
  - E =practical relative exposure time
- Ev = exposure-value
- f =focal length of lens
- HFD = hyperfocal distance, the "fixed focus" position which produces the greatest depth of field from infinity with an acceptable definition
  - m = magnification on film
- $R_1 = maximum$  distance beyond plane of accurate focus with acceptable definition
- $R_2$  = maximum distance in front of plane of accurate focus with acceptable definition
- $R_1 + R_2 = \text{depth of field}$ 
  - s = distance from lens to subject
  - t = actual exposure time in seconds
  - v = f-value; the diaphragm setting
  - V = effective f value

## 

MAGNIFICATION

- $m = \frac{f}{s f}$ [Equation #1]  $m = \frac{b - f}{f}$ [Equation #2]
- Example 1: f = 100 mm (Lens has focal length of 100 mm)
  - s = 150 mm (Subject is 150 mm in front of the lens) m = 100/150-100 = 2 (The image is
  - x 2 on the film for this given f and s)
- Example 2: With the same lens as in example 1 (f = 100 mm),
  - b = 150 mm (The film is 150 mm from the lens)
  - $m = \frac{150 100}{100} = \frac{1}{2}$ (The image is half the size of the subject)

- the difference between subject distance and focal length.
- The minimum magnification is achieved when s = infinity; the maximum magnification is achieved when s = f (although this would require a camera of infinite length).
- The magnification is directly proportional to the difference between the lens-to-film distance and the focal length of the lens.
- Example 3: f = 100 mm (The camera is focused on distant subjects at 100 mm)
  - For a magnification of x 2, the lens must be extended:

$$2 = \frac{B}{100}$$
;  $B = 200$  mm

For a magnification of x 8 (an increase of 4 times):

 $B = 4 \times 200 = 800 \text{ mm}$ 

For a magnification of x 10 (an increase of 5 times):

 $B = 5 \ge 200 = 1000 \text{ mm}$  (1 meter)

With the same lens, magnification increases in direct proportion to the bellows extension from the infinity position.

### 

DISTANCE FROM LENS TO FILM ("CAMERA LENGTH")

- b = f + B[Equation #3]
- b = fm + f = f (m + 1)[Equation #4]
- Example 1: f = 100 mm (Lens has focal length of 100 mm)
  - m = 2 (Magnification of x 2 desired)
- b = 100 (2 + 1) = 300 mm (Lens must be 300 mm from the film to give desired magnification)
- Example 2: f = 16 mm (Lens has focal length of 16 mm)
  - m = 2 (Magnification of x 2 desired) b = 16 (2 + 1) = 48 mm (Lens must be 48 mm from the film)

The "camera length" (distance from lens to film) is directly proportional to the focal length of the lens.

- The "camera length" is directly proportional to the magnification plus 1.
- The "camera length" cannot be less than the focal length of the lens (for the magnification would then have to be less than zero).
- For high magnifications, short focal length lenses are necessary for practical considerations in the length of the camera.

$$m = \frac{f}{s - f}$$
 (from Equation #1)

$$b = f \left(\frac{f}{s-f} + 1\right) = \frac{f^2}{s-f} + f$$

- The closer the subject, the longer the lens to film distance required for focus.
- Example 3: f = 16 (Lens of 16-mm focal length) b = 640 mm (Maximum lens to film distance possible with the bellows extended in the camera) What is the maximum magnification? b = f (m + 1), 640 = 16m + 16  $m = \frac{624}{16} = 3.9$  (The camera is too short to reach a magnification of x 4)
- The maximum extension of the camera determines the upper limit of magnification for a particular lens.

### 44444444444444444444444444

BELLOWS EXTENSION

 $B = b - f = \frac{f^2}{s - f}$ 

- Example 1: f = 48 mm (Lens focuses on distant subjects when b = 48 mm)
  - s = 96 mm (Subject 24 mm in front of the lens)

[Equation #5]

- $B = \frac{48 \times 48}{96 48} = 48 \text{ mm} \text{ (The lens must be ex$ tended by 48 mm to bring the $subject at 96 mm into focus)}$
- Example 2: With the same lens as in example 1 (f = 48 mm) and
  - s = 72 mm  $B = \frac{48 \times 48}{72 48} = 96 \text{ mm} \text{ (Lens must be extended} \text{ by 96 mm from the infinity position to bring the sub-ject into focus.}$
- The higher the magnification (the closer the subject to the lens), the longer the bellows extension.

$$m = \frac{f}{s - f} \quad (\text{from equation #1})$$
$$B = fm \qquad [Equation #6]$$

The distance of bellows extension is directly proportional to the magnification.

SUBJECT TO LENS DISTANCE

$$s = \frac{f}{m} + f = f(\frac{1}{m} + 1)$$
 [Equation #7]

Example 1: f = 100 mm m = x 4  $s = 100 (\frac{1}{4} + 1) = 125 \text{ mm}$  (Subject must be 125 mm from the lens to give magnification of x 4)

The higher the magnification desired, the closer the subject must be to the lens.

The lens can never focus on subjects closer than its focal length (for if m = infinity, then s = f).

$$b = fm + f \quad (\text{from equation #4})$$

$$s - b = \frac{f}{m} - fm$$

$$= \frac{f}{m} - fm + b = f \quad (\frac{1}{m} - m) + b \quad [\text{Equation #8}]$$

Example 2: For the same lens (f = 100 mm) and magnification (x 4) as in example 1, b = fm + f (from equation #4) b = (100 x 4) + 100 = 500 mm $s = \frac{100}{4} - 400 + 500 = 125 \text{ mm}$  (Subject must be 125 mm from the lens to give x 4)

$$m = \frac{b - f}{f} \quad (\text{from equation #2})$$

$$s = \frac{f}{m} + f = \frac{f^2}{b - f} + f = \frac{f^2}{B} + f \quad [\text{Equation # 9}]$$

\_\_\_\_\_

Example 3: f = 48 mm (Focal length of lens) b = 96 mm (Lens-to-film distance)

- $s = \frac{48 \times 48}{96 48} + 48 = 144 \text{ mm} (With a 48-mm lens)$ in a 96-mm camera, the subject comes into focus at 144 mm in front of the lens)
- The subject distance in front of the lens is proportional to the square of the focal length and inversely proportional to the difference between the camera length and the focal length of the lens.

f-VALUES ("STOPS")

s

The "stops" or settings of the diaphragm are values of

- [Equation #10]
- Stops or diaphragm settings are computed as the ratio of focal length to diameter of the lens opening.

Example 1: 
$$f = 100 \text{ mm}$$
  
 $d = 50 \text{ mm}$ 

v = f

 $v = \frac{100}{50} = 2$  (A very high *f*-value; a lens set at f/2 is "wide open" and many lenses are not provided with such a large opening)

Example 2: 
$$f = 48 \text{ mm}$$

d = 1 mm

 $v = \frac{48}{1} = 48$  (A very low *f*-value; a lens set at *f*/48 is "stopped way *down*." Note that high numbers for *v* are usually referred to as "low" *f*-values because a lens with that setting admits a low amount of light in a given interval)

For a fixed opening (d), the f-values vary in direct proportion to the focal length of the lens: the shorter the focal length, the smaller the f-value numbers for a given d. f/16for a lens with focal length of 48 mm (d = 3 mm) is the same size diaphragm opening as f/32 for a lens with focal length of 96 mm.

DIAMETER OF LENS OPENING

 $d = \frac{f}{d}$ 

[Equation #11]

- Example 1: f = 100 mm v = 32  $d = \frac{100}{32} = 3.1 \text{ mm}$ Example 2: f = 100 mm v = 32 $d = \frac{100}{15} = 6.3 \text{ mm}$
- For a given lens, the diameter of the lens opening is inversely proportional to the *f*-values ("settings").

EFFECTIVE f-VALUE

$$V = \frac{v \times b}{f}$$
 [Equation #12]

- Example 1: v = 22 (Diaphragm set at f/22) f = 48 mm b = 96 mm
  - $V = {22 \times 96 \over 48} = 44$  (With this lens-to-film distance, the camera functions as though it were set at f/44 when compared to its operation when focused at infinity)
- The higher the magnification (the longer the lens-to-film distance), the higher the effec-

tive *f*-value and the less light admitted to the film.

- The longer the focal length of the lens, the less the effective f-value.
- When the lens is focused at infinity, b = fand V = v.
- A short focal length lens will yield a higher effective f-value with the camera set for the same lens-to-film distance; since the magnification is higher, the effect is the same as closing the lens to a higher stop number.

$$V = \frac{v \ x \ b}{f} = \frac{v \ f(m + 1)}{f} \quad (\text{from equation #4})$$

$$V = v \ (m + 1)$$

$$e = (1 + m)^2 \quad (\text{see equation #23})$$

$$e = \frac{V^2}{v^2}$$

$$t_2 = \frac{(1 + m_2)^2}{(1 + m_1)^2} t_1 \quad (\text{see equation #24})$$

$$t_2 = (\frac{V_2^2}{V_1^2} \times \frac{v_1^2}{v_2^2}) \ t_1$$

- Exposure time varies with the square of the effective f-value divided by the square of the actual f-value.
- Example 2: In a camera, a 48-mm lens stopped down to f/16 is focussed at 96 mm from the film; proper exposure is found to be 10 seconds. What is the exposure time if a 16-mm lens stopped down to f/16 also is substituted (other factors constant)?

$$V_{1} = 16 \\ b_{1} = 96 \text{ mm} \quad V_{1} = \frac{16 \times 96}{48} = 32 \\ f_{1} = 48 \text{ mm} \\ v_{2} = 16 \\ b_{2} = 96 \text{ mm} \quad V_{2} = \frac{16 \times 96}{16} = 96 \\ f_{2} = 16 \text{ mm} \\ t_{2} = (\frac{96}{32} \times \frac{96}{32} \times \frac{256}{256}) 10 = 9 \times 10 = 90 \text{ seconds}$$

The same solution could also be derived from the magnifications:

$$m_{1} = \frac{b-f}{f} = \frac{96-48}{48} = 1$$

$$m_{2} = \frac{96-16}{16} = 5$$

$$t_{2} = \frac{(1+m_{2})^{2}}{(1+m_{1})^{2}}t_{1} = \frac{(6)^{2}}{(2)^{2}} = 10 = 90 \text{ seconds.}$$

### 

DEPTH OF FIELD

Depth of field =  $R_1 + R_2$ , where  $R_1 = \frac{CS}{md - c}$ , and [Equation #13]  $R_2 = \frac{CS}{md + c}$  [Equation #14]

Example 1: c = 0.1 mm (Acceptable circle of confusion of 0.1 mm on film) f = 100 mm (Focal length of lens) s = 3280 mm (Subject 3.28 meters from lens) v = 4.5 (Diaphragm setting at f/4.5)  $d = \frac{f}{4.5} = \frac{100}{4.5} = 22.2 \text{ mm}$  $m = \frac{f}{s - f} = \frac{100}{3280 - 100} = \frac{1}{31.8}$  $md = \frac{22.2}{31.8} = 0.70$  $R_{I} = \frac{0.1 \times 3280}{0.70 - 0.1} = \frac{328}{0.6} = 546 \text{ mm}$  $R_2 = \frac{0.1 \times 3280}{0.70 + 0.1} = \frac{328}{0.8} = 410 \text{ mm} \text{ (Subject in acceptable focus}$ from 3280 - 410 mm to 3280 + 546 mm, or from 2870 mm to 3826 mm) Depth of field = 546 + 410 = 956 mm at this s, m, and d.  $s = f(\frac{1}{m} + 1)$  (from equation #7)  $R_{I} = \frac{cf(\frac{1}{m} + 1)}{m(\frac{f}{v}) - c}$  (from equation #11) (Equal [Equation #15] Example 2: c = 0.1 mm m = 2f = 96 mm v = 16

$$R_{I} = \frac{0.1 \times 96(0.5 + 1)}{2(6) - 0.1} = \frac{14.4}{11.9} = 1.21 \text{ mm}$$

$$R_{2} = \frac{14.4}{12.1} = 1.19 \text{ mm}$$

$$R_{1} + R_{2} = 2.40 \text{ mm} \text{ (Depth of field)}$$

Example 3: Keeping the same acceptable circle of confusion, magnification, and diaphragm opening, but using a lens with

$$f' = 16 \text{ mm}$$

$$R_{I} = \frac{0.1 \times 16(0.5 + 1)}{2(1) - 0.1} = \frac{2.4}{1.9} = 1.26 \text{ mm}$$

$$R_{2} = \frac{2.4}{2.1} = 1.14 \text{ mm}$$

$$R_{I} + R_{2} = 2.40 \text{ mm}$$

Comparing examples 2 and 3, we note that

For a given magnification and f-value, the depth

of field is the same regardless of the focal length of the lens used. A lens of long focal length has the identical depth of field as a lens of short focal length when used at the same f-stop to produce the same magnification of image. Any difference in depth of field at the same magnification must be due to the exactitude with which the lens is ground and polished.

For practical purposes,

Depth of field =  $R_1 + R_2 = \frac{2cs}{md}$  [Equation #16]

Example 4: Using the same set-up as in example 1, Depth of field =  $\frac{2 \times 0.1 \times 3280}{0.70} = \frac{656}{.70} = 937$ 

Example 5: Using the same set-up as in example 2. Depth of field =  $\frac{28.8}{12}$  = 2.40 mm

If the subject is fairly close to the lens, md is large compared to c and the depth of field can be approximated as 2cs/md.

## 444444444444444444444444444444444

HYPERFOCAL DISTANCE

H

$$FD = \frac{fd}{c}$$
 [Equation #17]

This formula is derived as follows:

$$R_{l} = \frac{cs}{md - c} \quad (\text{from equation #13})$$
  
At  $R_{l} = \text{infinity}, \ md - c = 0 \text{ and } md = c.$ 
$$m = \frac{f}{c - f} \quad (\text{from equation #1})$$

For practical purposes, s - f = HFD, since f is insignificant compared to the subject distance involved. Hence,

$$m = \frac{f}{HFD}$$
$$md = c = \frac{fd}{HFD}$$
$$HFD = \frac{fd}{c}$$

Example 1: 
$$f = 100 \text{ mm}$$
  
 $d = f/16 = 100/16$   
 $c = 0.1 \text{ mm}$   
100 x 100 coro mm (100 x 100 coro mm)

$$HFD = \frac{1}{0.1 \times 16} = 6250 \text{ mm} = 6.25 \text{ meters}$$

$$R_2 = \frac{cs}{md + c} = \frac{0.1 \times 0250}{(\frac{100}{6150} \times \frac{100}{16}) + 0.1} = \frac{0.23}{.202} = 3120 \text{ mm}$$

Hence, objects are in acceptable focus from 3.13 meters to infinity with this lens set at f/16 and focused accurately at 6.25 m.

The hyperfocal distance decreases (the lens produces a greater depth of coverage) if the lens has a shorter focal length. However, it must be remembered (see equation #1) that a lens of shorter focal length also produces a smaller image (m) on the film. This is the principle on which the mini-cameras are designed; if the focal length of the lens is very short, the camera has nearly universal focus.

- If we accept a larger circle of confusion, say 0.25 mm, the hyperfocal distance decreases. The image will be "fuzzier" at infinity and at the  $R_2$  position, but will cover a greater span of distance.
- Decreasing the aperture (d) will give closer hyperfocal distance and increase the span in acceptable focus. Hence, with a fast film, short focal length lens, and small f-stop, photographs could be taken with a fixed-focus camera that would give acceptable definition from less than a meter to infinity; the drawback is the small size of the image produced.
- Once calculated, the hyperfocal distance can be used to find the depth of field, thus:

$$R_{1} = R_{2} = \frac{cs}{md} \quad (\text{from equation #16})$$

$$HFD = fd/c \quad (\text{from equation #17})$$

$$\frac{cs}{md} = \frac{sf}{m} \times \frac{c}{fd} = R = R = \frac{sf}{m \times HFD}$$
[Equation

Another useful formula for depth of field involving hyperfocal distance is derived:

#18]

$$m = \frac{J}{s - f} \quad (\text{from equation #1})$$

$$R_1 = R_2 = \frac{sf}{m \ \text{HFD}} = \frac{sf}{\frac{f}{s - f} \ (\text{HFD})} =$$

$$R_1 = R_2 = \frac{s(s - f)}{\text{HFD}} \quad [\text{Equation #19}]$$

Still another, using f, m, and HFD:

$$s = \frac{f}{m} + f \quad (\text{from equation } \#7)$$

$$R_1 = R_2 = \frac{\frac{f}{m} \left(\frac{L}{m} + f\right)}{HFD} \qquad [Equation # 20]$$

Example 2: Using the same set-up as in example
2 under "Depth of Field":

$$c = 0.1 \text{ mm} \qquad m = 2$$
  

$$f = 96 \text{ mm} \qquad d = 6 \text{ mm}$$
  

$$HFD = \frac{fd}{c} = \frac{96 \times 6}{0.1} = 5760$$
  

$$s = \frac{f}{m} + f = 48 + 96 = 144 \text{ mm}$$
  

$$R_1 = R_2 = \frac{\frac{f}{m}(\frac{f}{m} + f)}{HFD} =$$

$$\frac{\frac{96}{2}(\frac{96}{2}+96)}{5760} = \frac{48(144)}{5760} = \frac{6912}{5760} = 1.20 \text{ mm},$$

Or solved as

$$R_1 = R_2 = \frac{sf}{m \ HFD} = \frac{96 \ x \ 144}{2 \ x \ 5760} = 1.20 \ mm.$$

Example 3: Using the same set-up as in example
3 under "Depth of Field":

$$c = 0.1 \text{ mm}$$
  $m = 2$   
 $f = 16 \text{ mm}$   $d = 1 \text{ mm}$   
 $\frac{fd}{c} = \frac{16 \text{ x } 1}{0.1} = HFD = 160 \text{ mm}$ 

Using equation #19 --

$$R_{I} = R_{2} = \frac{\prod_{m} (\prod_{m} + f)}{HFD} = \frac{8(8 + 16)}{160} = 1.20 \text{ mm}$$

A more accurate approximation of the depth of field is:

$$R_{I} = \frac{sf}{m \ HFD - f} \quad \text{and} \qquad [Equation \ #21]$$

$$R_{2} = \frac{sf}{m \ HFD + f} \qquad [Equation \ #22]$$

Example 4: Using the same set-up as in example 3 under "Depth of Field".

$$c = 0.1 \text{ mm} \qquad m = 2$$

$$f = 16 \text{ mm} \qquad d = 1 \text{ mm}$$

$$FD = 160 \text{ mm}$$

$$R_I = \frac{24 \text{ x } 16}{320 - 16} = \frac{384}{304} = 1.26 \text{ mm}$$

$$R_2 = \frac{24 \text{ x } 16}{320 + 16} \qquad \frac{384}{336} = 1.14 \text{ mm}$$

As the hyperfocal distance decreases, the depth of field becomes greater.

## *444444444444444444444444*

RELATIVE EXPOSURE TIME

For all magnifications, relative exposure  $e = (1 + m)^2$ [Equation #23] Example 1: For subjects at infinity (m = 0) the exposure is 1/50 second.  $e = (1 + 0)^2 = 1$ For natural size on negative (m = 1) with the same light,  $e = (1 + 1)^2 = 4$  (The exposure is 4 times as long, and the time will be  $4 \times 1/50 = 2/25$  second) The formula can be written for actual time as:  $t_2 = \frac{(1 + m_2)^2}{(1 + m_1)^2} t_1$ [Equation #24] Example 2:  $m_1 = 2$  and  $t_1 = 10$  (Exposure is 10 seconds for magnification of x 2)

				Ch	anging	to ma	gnific	ation	of			
		x 0.5	<b>x</b> 0.75	x l	x 1.5	x 2	x 3	x 4	x 5	хб	x 8	x 10
	x 0.5		2.25	4	9	16	36	64	100	144	256	400
4	x 0.75	.455		1.78	4	7.12	16	28.5	44.6	64	114	178
5	хl	.250	.562		2.25	4	9	16	25	36	64	100
5	x 1.5	.111	.250	.445		1.78	4	7.12	11.2	16	28.5	44.6
	x 2	.063	.140	.250	.563		2.25	4	6.25	9	16	25
١	х 3	.028	.063	.111	.250	.445		1.78	2.78	4	7.12	11.2
	x 4	.016	.035	.063	.140	.250	.563		1.56	2.25	4	6.25
	x 5	.010	.022	.040	.090	.160	.360	.640		1.44	2.56	4
	<b>x</b> 6	.007	.016	.028	.063	.111	.250	.445	.700		1.78	2.78
	x 8	.004	.009	.016	.035	.063	.141	.250	.390	.563		1.56
	x 10	.003	.006	.010	.023	.040	.090	.160	.250	.360	.640	

PRACTICAL EXPOSURE GUIDE FOR CHANGING MAGNIFICATIONS

Example 1: Correct exposure at x 1 is 3 seconds. Exposure at x = 8

 $64 \times 3 = 192$  seconds (1 min 12 sec)

Example 2: Correct exposure at x 4 is 50 secs. Exposure at x 0.5 =

 $.016 \times 50 = 0.8$  second 

For magnification x 5 with the same lens, light, and f-stop:

$$m_2 = 5$$

$$t_2 = \frac{(1+5)}{(1+2)} \times 10 = \frac{36}{9} \times 10 = 40 \text{ seconds}$$

The relative exposure is proportional to the square of the magnification + 1.

#### PRACTICAL EXPOSURE TIME

For changing magnifications at natural size and above, it is better to compute exposure as:

$$E = m^{2}k \text{ and } [Equation #25]$$

$$t_{2} = \frac{m^{2}}{m_{1}^{2}} t_{1}$$

Example 1:  $m_1 = 2$  and  $t_1 = 6$  seconds.  $m_2 = 8$  $t_2 = \frac{8}{2} \ge 6 = \frac{64}{4} \ge 6 = 96$  seconds.

For magnifications at natural size and above,

Example 3: Correct exposure at x 10 is 1 minute. Exposure at x = 2 $.04 \times 60 = 2.4$  seconds

Example 4: Correct exposure at x 0.5 is 1/25 second. Exposure at x 8 =

 $256 \times .04 = 10.24$  seconds

the exposure time in changing magnifications is increased or decreased by the ratio of the square of the new magnification to the square of the old magnification.

If the magnification remains constant, the time changes with the f-value changes, as

$$\frac{7^2}{7_1^2} \times t_1 \qquad [Equation \# 26]$$

Example 2:  $t_1 = 10$  seconds

 $t_2 =$ 

$$v_1 = 11$$
 (d = f/11)  
 $v_2 = 16$  (d = f/16)

$$t_2 = \frac{256}{121} \times 10 = 21.1$$
 (about 21 seconds).

- Regardless of the focal length of the lens used, the time of exposure increases or decreases by the ratio of the square of the new f-value to the square of the old f-value.
- The higher the f-value (the smaller the diameter of the diaphragm opening), the longer the exposure time.

PRACTICAL EXPOSURE GUIDE FOR CHANGING f-VALUES

						Changi	ng to	f/					
	2.8	3.5	4	4.5	5.6	6.3	8	9.5	11	16	22	32	45
2.8	3	1.5	2	3	4	6	8	12	16	32	64	128	256
3.5	.667		1.50	2	3	4	6	8	12	24	48	96	192
4	.500	.667		1.50	2	3	4	6	8	16	32	64	128
4.5	.333	.500	.667		1.50	2	3	4	6	12	24	48	96
چ 5.6	.250	.333	.500	.667		1.50	2	3	4	8	16	32	64
E0.3	.167	.250	.333	.500	.667		1.50	2	3	6	12	24	48
д 8 Н	.125	.167	.250	.333	.500	.667		1.50	2	4	8	16	32
лі 9 <b>.</b> !	.083	.125	.167	.250	.333	.500	.667		1.50	3	6	12	24
LL Cha	.063	.083	.125	.167	.250	.333	.500	.667		2	4	8	16
16	.031	.042	.063	.083	.125	.167	.250	.333	.500		2	4	8
22	.016	.021	.031	.042	.063	.083	.125	.167	.250	.500		2	4
32	.008	.010	.016	.021	.031	.042	.063	.083	.125	.250	.500		2
45	.004	.006	<b>.0</b> 08	.010	.016	.021	.031	.042	.063	.125	.250	.500	
	1												

Example 1: Correct exposure at f/4.5 is 3 secs. Exposure at f/22 = $24 \times 3 = 72$  seconds

Example 2: Correct exposure at f/5.6 is 8 secs. Exposure at f/16 =

Example 3: Correct exposure at f/4 is 1/50 sec. Exposure at f/45 = $128 \times .02 = 2.56$  seconds Example 4: Correct exposure at f/32 is 40 secs.

 $.042 \times 40 = 1.68$  seconds

Exposure at f/6.3 =

 $8 \times 8 = 64$  seconds 

EXPOSURE-VALUE

This factor relates the light admitted during an exposure

$$\frac{t}{v^2}$$

to the speed of the film (ASA).

$$\frac{t}{v^2} = \frac{25}{ASA \times 2^{Ev}}$$
 [Equation #27]

Ev is an exponent of 2 in the formula. It can be shown to be:

> $2^{E\nu} = \frac{25 \times v^2}{t \times ASA}$ [Equation #28]

- $Ev \ge \log 2 = \log 25 + 2 \log v \log t \log ASA$  $Ev = \frac{\log 25 + 2 \log v - \log t - \log ASA}{2}$ log 2
- Ev measures the quantity of light necessary to produce a given effect on the film being used in the camera.

- Briefly, Ev acts as a value on an exposure meter adjusted for the film speed. Actually some exposure meters are calibrated in Ev numbers.
- With the f-value (v) and time (t) remaining constant, Ev varies with the negative logarithm of the film speed (ASA). The higher the ASA rating, the lower the Ev (other factors being constant); this is logical to reason through, since with a fast film less light is needed to produce the same density of registration on the film.
- With the same film speed (ASA) and f-value (v), the necessary Ev for proper exposure is greater with less time (t).
- With the same film (ASA) and time (t), the necessary Ev for proper exposure is greater with increased f-value (v), that is, with smaller diaphragm opening,

									_		
Ev	2 <sup>Ev</sup>	Ev	2 <sup>Ev</sup>	Ev	2 <sup>Ev</sup>	Ev	2 <sup>E</sup> <sup>ν</sup>	Ev	2 <sup>Ev</sup>	Ev	<u>2 <sup>E</sup>v</u>
0.1	1.072	-8	.0039	-3	.125	2	4.00	7	128	12	4096
0.2	1.149	-7.5	.0055	-2.5	.194	2.5	5.66	7.5	181	12.5	5793
0.3	1.231	-7	.0078	-2	.250	3	8.00	8	256	13	819 <b>2</b>
0.4	1.320	-6.5	.0111	-1.5	.354	3.5	11.31	8.5	362	13.5	11585
0.5	1.414	-6	.0156	-1	.500	4	16.00	9	512	14	16384
0.6	1.516	-5.5	.0221	-0.5	.705	4.5	22.63	9.5	724	14.5	23170
0.7	1.625	-5	.0313	0	1.00	5	32.00	10	1024	15	<b>32</b> 768
0.8	1.741	-4.5	.0442	0.5	1.41	5.5	45.26	10.5	1448	15.5	46340
0.9	1.866	-4	.0625	1	2.00	6	64.00	11	<b>204</b> 8	16	65536
1.0	2.000	-3.5	.0885	1.5	2.83	6.5	90.51	11.5	2896		

TABLE OF  $2^{E_{\nu}}$  VALUES

From this table other  $2^{E\nu}$  values can be readily computed, since

$$2^{E\nu_1 + E\nu_2} = 2^{E\nu_1} \times 2^{E\nu_2}$$

Thus,  $2^{20} = 2^{10} \times 2^{10} = 1024 \times 1024 = 1048576$ .

And  $2^{7.2} = 2^{7.0} \times 2^{0.2} = 128 \times 1.149 = 147.1$ 

more convenient to have the value of  $2^{E_{\nu}}$  for

Intensity of light is porportional to  $2^{Ev}$  and

not to Ev. Thus, if one light bulb produces an

Ev = 5, then the concentrated output of 5 bulbs

 $32 \times 5 = 160 = 2^{Ev}$ , and Ev = about 7.32.

Example 4: In the same set-up as in example 3, what would be the time at f/22 for a film of ASA = 25 with the same lighting?

reference).

is calculated from  $2^{E_{\nu}}$  values as:

$$t_{2} = \frac{v_{2}^{2} \times 25}{ASA_{2} \times 2^{E_{\nu}}} \text{ and}$$
  

$$t_{1} = \frac{v_{1}^{2} \times 25}{ASA_{1} \times 2^{E_{\nu}}}$$
  

$$t_{2} = \frac{v_{2}^{2} \times ASA_{1}}{v_{1}^{2} \times ASA_{2}} t_{1}$$
 [Equation #30]

If the lighting of the photographic set-up in example 3 remains constant and the film and f-value are changed, then

$$t_2 = \frac{484 \times 100}{64 \times 25} \times 10 = 301$$
 seconds = about 5 minutes.

- Other factors being equal, the exposure time is inversely proportional to the speed of the film.
- Example 5: In the set-up of example 4, what would be the proper diaphragm setting to reduce the exposure to 1 minute?

Example 1: Proper negative density is obtained on film of ASA = 25 at 1 second with aperture set at f/16. What is the quantity of light admitted during exposure?

 $2^{E_{\nu}} = \frac{25 \times v^{2}}{t \times ASA}$  (From equation #28)  $2^{E_{\nu}} = \frac{25 \times 256}{1 \times 25} = 256; \text{ from the table, } E_{\nu} = 8.$ 

Example 2: The light source is found to have an Ev = 12 at the magnification being used. What would be the proper exposure time for film of ASA = 64 at f/22?

$$Ev = 12 \qquad 2^{Ev} = 4112 v = 22 \qquad v^2 = 484 t = \frac{v^2 \times 25}{ASA \times 2Ev} \qquad [Equation #29] t = \frac{484 \times 25}{64 \times 4096} = \frac{12100}{262144} = \frac{1}{25} \text{ second.}$$

- Example 3: Proper exposure is found to be 10 seconds at f/8 on film of ASA = 100. What is the rating of the light source in this set-up (to be used for future reference)?
  - $2^{E_{\nu}} = \frac{25 \times 64}{100 \times 10} = \frac{1600}{1000} = 1.6; \text{ from the table,}$ Ev = about 0.6. (For most usages, it is

2 <sup><i>E</i>v</sup>	Ev	2 <sup><i>Ev</i></sup>	Ev	2 <sup><i>Ev</i></sup>	Ev	2 <sup><i>Ev</i></sup>	Ev	2 <sup>Ev</sup>	Ev	2 <sup>.Ev</sup>	Ev
.01	-6.644	.55	8625	3.0	1.585	20	4.322	95	6.570	850	9.731
.02	-5.644	.60	7369	3.5	1.807	22	4.459	100	6.644	900	9.831
.03	-5.059	.65	6215	4.0	2.000	24	4.585	110	6.781	1000	9.966
.04	-4.644	.70	5146	4.5	2.170	26	4.700	120	6.907	1100	10.103
.05	-4.322	.75	4150	5.0	2.322	28	4.807	130	7.022	1200	10.229
.06	-4.059	.80	3219	5.5	2.459	30	4.907	140	7.129	1300	10.344
.07	-3.836	.85	2345	6.0	2.585	32	5.000	150	7.229	1400	10.451
.08	-3.644	.90	1520	6.5	2.700	34	5.087	160	7.322	1500	10.550
.09	-3.474	.95	0740	7.0	2.807	36	5.170	170	7.409	1600	10.644
.10	-3.322	1.0	0.0000	7.5	2.907	38	5.248	180	7.492	1700	10.731
.12	-3.059	1.1	+.1375	8.0	3.000	40	5.322	190	7.570	1800	10.813
.14	-2.833	1.2	.2630	8.5	3.087	42	5.392	200	7.644	1900	10.892
.16	-2.644	1.3	.3785	9.0	3.170	44	5.459	250	7.966	2000	10.966
.18	-2.474	1.4	.4854	9.5	3.248	46	5.523	300	8.229	2200	11.103
.20	-2.322	1.5	.5850	10	3.322	48	5.585	350	8.451	2400	11.229
.22	-2.184	1.6	.6781	11	3.460	50	5.644	400	8.644	2600	11.344
.24	-2.059	1.7	.7655	12	3.585	55	5.781	450	8.814	2800	11.451
.26	-1.943	1.8	.8480	13	3.700	60	5.907	500	8.965	3000	11.550
.28	-1.836	1.9	.9260	14	3.807	65	6.022	550	9.103	3500	11.773
.30	-1.737	2.0	1.0000	15	3.907	70	6.129	600	9.229	4000	11.966
.35	-1.515	2.2	1.138	16	4.000	75	6.229	650	9.344	4500	12.136
.40	-1.322	2.4	1.263	17	4.088	80	6.322	700	9.451	5000	12.286
.45	-1.152	2.6	1.378	18	4.170	85	6.409	750	9.551	5500	12.425
.50	-1.000	2.8	1.485	19	4.248	90	6.492	800	9.644	6000	12.551

TABLE OF EV VALUES

The simplest solution is to refer to the "Practical Exposure Guide for Changing f-values." In this chart, the f-value having the equivalent of 1/5 or 0.2 is slightly below f/11 (read from f/22).

#### FILMS AND PAPERS

Of all photographic phenomena, the latent image is one of the most fascinating -- an invisible picture retained in the emulsion from the moment of exposure until the beginning of development. This amazing potential of the film or printing paper cannot be seen in any way, yet there it is, awaiting chemical reactions to make its image "real." How is this latent image re-

_	and the second division in which the second division is not the second division of the second division is not the second division of the										
				FOR	CIRCLE OF	F CONFUSI	ON = 0.1	mm			
		x 0.5	x 0.75	хl	x 2	х 3	x 4	х б	x 8	x 10	x 20
ЪС	f/8	9.60	3.73	3.20	1.20	.71	.50	.31	.23	.18	.08
ttiı	<i>f/</i> 11	13.20	5.13	4.40	1.65	.98	.69	.43	.31	.24	.12
l se	<i>f/</i> 16	19.20	7.47	6.40	2.40	1.42	1.00	.62	.45	.35	.17
ragm	f/22	26.40	10.27	8.80	3.30	1.96	1.38	.86	.62	.48	.23
aph:	<i>f/</i> 32	38.40	14.93	12.80	4.80	2.84	2.00	1.24	.90	.70	.34
Dİ	f/45	54.00	21.00	18.00	6.75	4.00	2.81	1.77	1.28	.99	.47
_											
				FOR	CIRCLE OF	F CONFUSI	ON = 0.2!	5 mm			
		x 0.5	x 0.75	FOR × 1	CIRCLE OF x 2	F CONFUSI x 3	ON = 0.23 $x 4$	5mm x6	x 8	x 10	x 20
Б.	<i>f/</i> 8	x 0.5 24.00	x 0.75 9.33	FOR × 1 8.00	CIRCLE OF x 2 3.00	F CONFUSI x 3 1.78	ON = 0.25 x 4 1.25	5 mm × 6 .78	× 8 .56	x 10	× 20 .21
cting	f/8 f/11	x 0.5 24.00 33.00	x 0.75 9.33 12.83	FOR × 1 8.00 11.00	CIRCLE OF x 2 3.00 4.13	F CONFUSI x 3 1.78 2.44	ON = 0.2! x 4 1.25 1.72	5 mm x 6 .78 1.07	× 8 .56 .77	x 10 .44 .61	× 20 .21 .29
setting	f/8 f/11 f/16	x 0.5 24.00 33.00 48.00	x 0.75 9.33 12.83 18.67	FOR x 1 8.00 11.00 16.00	CIRCLE OF x 2 3.00 4.13 6.00	F CONFUSI x 3 1.78 2.44 3.56	$ON = 0.25 \\ \times 4 \\ 1.25 \\ 1.72 \\ 2.50 $	5 mm x 6 .78 1.07 1.56	× 8 .56 .77 1.12	x 10 .44 .61 .88	x 20 .21 .29 .42
agm setting	f/8 f/11 f/16 f/22	x 0.5 24.00 33.00 48.00 66.00	x 0.75 9.33 12.83 18.67 25.67	FOR x 1 8.00 11.00 16.00 22.00	CIRCLE OF x 2 3.00 4.13 6.00 8.25	F CONFUSI x 3 1.78 2.44 3.56 4.88	$ON = 0.22 \\ \times 4 \\ 1.25 \\ 1.72 \\ 2.50 \\ 3.44$	5 mm x 6 .78 1.07 1.56 2.14	× 8 .56 .77 1.12 1.55	x 10 .44 .61 .88 1.21	x 20 .21 .29 .42 .58
aphragm setting	f/8 f/11 f/16 f/22 f/32	x 0.5 24.00 33.00 48.00 66.00 96.00	x 0.75 9.33 12.83 18.67 25.67 37.33	FOR x 1 8.00 11.00 16.00 22.00 32.00	CIRCLE OF x 2 3.00 4.13 6.00 8.25 12.00	F CONFUSI x 3 1.78 2.44 3.56 4.88 7.11	$ON = 0.22 \\ x 4 \\ 1.25 \\ 1.72 \\ 2.50 \\ 3.44 \\ 5.00$	5 mm x 6 .78 1.07 1.56 2.14 3.11	x 8 .56 .77 1.12 1.55 2.25	x 10 .44 .61 .88 1.21 1.76	× 20 .21 .29 .42 .58 .84

TABLE OF THEORETICAL DEPTHS OF FIELD (in mm)

Computed by formula for lenses of equal and average accuracy:  $2cs = \frac{2c(\frac{1}{m} + 1)}{2cv (\frac{1}{m} + 1)} = \frac{2cv (\frac{1}{m} + 1)}{m}$ 

Depth of field = 
$$\frac{2CS}{md} = \frac{m}{m/y}$$
 =

corded? We can only describe it in general, because even the experts in physical chemistry seem to be lacking a clear explanation.

Physical properties. -- Films and papers contain three essentials: emulsion, halides, and support.

EMULSION AND HALIDES. Film and paper are photographically sensitive to light because they contain, suspended in the gelatin coating, millions of tiny crystals of silver halides. These halides change after exposure to light and thereupon become capable of being changed to metallic silver (which is visible) whenever immersed in a chemical solution known as the developer or reducing agent. This physical change of the crystals upon exposure to light is the whole basis of photography. Yet it remains a mysterious reaction.

The silver halide crystals in the emulsion

are exposed in proportion to the intensity of the light striking the film. Bright light (reflected from a white area of the subject) will expose a thick layer of halides by penetrating the translucent emulsion; dim light (from dark or gray areas of the subject) will expose a very thin layer; and the light striking a black area of the subject may be almost entirely absorbed, so that the faint light reflected from it may not affect the halides at all.

The developer changes these exposed halides into black metallic silver to form the visible image. The brightest parts of the subject (which reflected light with the strongest intensity) will be represented by the darkest part of the developed image, and, conversely, darkest or shadow parts of the subject (which reflected light of low intensity) will be represented by the lightest ("thinnest") areas of the image. This reversal of dark-for-light and light-fordark makes the film a *negative*. When a negative is printed on photographic paper, the tones are again reversed in a repetition of the process, and the tones correspond to those in the original subject; such a print is called a *positive*.

SUPPORT OR BASE. Without a sheet for support, the gelatinous emulsion would collapse, fold, and roll into a useless mass during the developing process. The material used as the *support* or *base* for the photographic emulsion must have two essential qualities: (1) it must be photographically inert (incapable of causing any harmful action to the latent image or the developing image), and (2) it must remain unaffected by the chemical solutions used in processing.

The base for films is usually some form of cellulose derivative which is transparent, tough, lightweight, and flexible, such as cellulose acetate. For photographic prints, paper is the ideal support, for it is durable, fairly flexible, and opaque (as well as economical). Films are made in different grain-size emulsions and numerous speeds. Printing paper is made in several thicknesses (weights), different textures, and a standard series of contrasts.

EMULSION. Photographic emulsions are of two general kinds according to their sensitivity to light. Negative emulsions are very sensitive and used in the camera, and positive emulsions are much less sensitive and used for printing the negative.

In most emulsions for negatives, a small percentage of potassium iodide (KI) is added to the silver bromide (AgBr) to create crystals of silver bromoiodide (Ag<sub>2</sub>BrI), which are much more sensitive to light than the pure silver bromide. Emulsions for printing papers contain crystals of either silver chloride (AgCl) or silver bromide (AgBr) or a combination of the two; for enlarging, the faster "bromide" papers are used.

Gelatin is the ideal substance for keeping these silver halides in suspension because it swells when wet, allowing penetration of the processing solutions, and contracts when dried, keeping the grains in their original positions. Gelatin requires no special treatment below 85°



Section through a sheet of film.

F, but above that temperature the films must be put into a hardening bath.

OVERCOATING. To prevent scratches and abrasions, most film emulsions have a protective overcoating of clear hardened gelatin. Paper emulsions are often protected similarly because of the thinness of their emulsion layer.

NONCURL BACKING. Because the emulsion swells as it is wetted and shrinks as it is dried, while the film base remains unaffected, the film tends to curl one way and then the other and may permanently warp in the process. Modern films alleviate this problem by having a gelatin backing (on the side of the film base opposite to the emulsion) that swells and shrinks at the same rate as the emulsion layer and hence counteracts the strains of expansion and contraction.

ANTIHALATION BACKING. A dye added to the noncurl backing absorbs any intense light that penetrates the emulsion. If not absorbed, such light rays would reflect from the back of the film, scatter, and produce a halo effect around the bright spots of the image. The color of the antihalation dye is either a neutral gray or the color to which the film is sensitive.

Because the dye is highly soluble in water, it is completely removed during early stages of development and never poses a problem in printing.

Emulsion properties. -- Certain properties or characteristics are built into a film during manufacture. These are so varied that every film company makes numerous different types in every size of film. The properties include film speed, inherent contrast, color sensitivity, latitude, graniness, and resolving power. For a particular photographic task, one or two films will offer a much better combination of the desired characteristics than any of the others. From the following factors, you can make a logical choice.

FILM SPEED. No other property is as much emphasized in advertising as that of speed. Even in photomicrography, where exposures are commonly measured in seconds instead of fractions of a second, being able to shorten the time is something of an advantage. This advantage is not so overwhelming, however, that it should outweigh other factors. A few extra seconds are not so important that we should sacrifice the best contrast or grain size. After all, the specimen is motionless, and fast shutter speeds and film are hardly required to "freeze" the action.

Film speed expresses the sensitivity of the emulsion to light. The faster the film, the less amount of light needed to produce a satisfactory negative. Speed starts with the way the emulsion is "cooked" or "ripened" before it is coated onto the backing of the film. The longer the emulsion is processed before being spread onto the film, the larger the grains of silver bromide, the greater their sensitivity, and the faster the film speed.

Most films are judged by standards set by the American Standards Association -- the ASA rating. This is based on the minimum exposure required for a good negative, and can be used for direct comparison of films made by different cimpanies. The numbers in the ASA rating are called *exposure indices* or *film speed*, and are used in connection with exposure meters or exposure computations. A film with ASA of 100 is twice as fast as one with ASA of 50 and would require half as much exposure time to produce a negative of the same density.

Many films have two ASA ratings, one for daylight and one for tungsten (artificial) light. Daylight contains much more blue (to which nearly all films are extremely sensitive) than does tungsten light. Therefore, the tungsten speed rating is much lower than the daylight rating, because the film will need an appreciably longer exposure with the blue-less artificial light.

GRAIN.' Grain size is closely related to film speed. The "ripening" process which increases sensitivity also increases the size of the grains by causing them to "clump" together. High-speed films, therefore, have inherent graininess. When such grains are much enlarged, the print shows visible (discernible) "grain" that destroys the sharpness of the image. For enlargements, the slower-speed films are to be preferred because of their small grain.

The main factor in graininess of the negative is the inherent grain size traced back to its manufacture, but the development of the negative can also increase the grain size. The socalled "fine-grain" developers control the grain size to a certain extent by preventing excessive clumping together of silver grains during processing.

COLOR SENSITIVITY. Because silver bromide is sensitive to blue, violet, and ultraviolet, all photographic emulsions are inherently sensitive to these wave lengths. Sensitivity to other colors is made possible by adding various sensitizing dyes to the emulsion during manufacture to achieve the balance desired.

The human eye and the film differ. The human eye sees both brightness and color, but the film "sees" only degrees of brightness (in the black-and-white films used in photomicrography for reproduction in publication) in the range of wavelengths to which it is sensitive. Actually, with selected dyes the film can be made more sensitive than the human eye, discerning shorter wave lengths than violet and/or longer wave lengths than red. According to color sensitivity, films can be divided into three general classes:

Orthonon (colorblind) film. Because of its limited sensitivity (blue, violet, ultraviolet), this film is not now used as much as others. It is slow in speed, fine-grained, and very high in contrast. It was used to a great extent for copying black-and-white drawings and charts.

Orthochromatic film. Hailed as the "true color" sensitive film, this had a greater range because the dye made it also sensitive to green. The red colors did not register, however, and any reddish areas were little represented in the negative and came out black in the print. The advantage of this film is that it can be processed under a dark red safelight, and for that reason it is popular with amateurs. The film has fine grain, medium contrast, and medium speed and hence serves for a variety of situations.

Panchromatic film. With additional dyes, film was manufactured to be sensitive to red, in addition to the other colors. Because its coverage corresponds roughly to the spectrum visible to the human eye, this film yields the widest color response and most natural-looking interpretation of colors in tones of the blackand-white scale.

Films having fairly even balance of sensitivity to red, green, and blue are called type B panchromatic. Those with higher red sensitivity are called type C, and are used with artificial lighting, since tungsten is strong in the red color.

LATITUDE. All subjects are composed of points or areas of differing brightness. The brightest areas are termed the *highlights*, the darkest areas the *shadows*, and all areas intermediate are called *halftones*. The ability of a film to record the range of brightness values is the *film latitude*. A film capable of producing a long range of these values, from darkest shadow to brightest highlight, has a wide latitude. But a film that can produce only a short range of brightness values has small latitude, with little within its halftone range.

The average panchromatic film of medium contrast has a latitude of 1 to 130; that is, the highlight can be 130 times as bright as the shadow area and the film will still record both and all gradations of tone between.

Latitude in black-and-white photography allows a degree of underexposure or overexposure and still gives satisfactory prints. This degree of deviation from the correct exposure which will still result in a "good" negative is called *acceptable latitude*.

Inasmuch as the photomicrographer will probably work out a more or less "standard" set-up, with the same lighting, lens, magnification, and film for numerous photographs, latitude will not be as important a factor for him as it would be for the photographer taking each shot with different lighting and magnification. Once his results show good depth and clarity, he can keep on shooting with no particular need to worry about over- or underexposure. The only variable which can affect the exposure in his standard set-up will be the thinness of the white coating of sublimated ammonium chloride, which can give dimmer or brighter reflection with the same lighting.

CONTRAST. The difference between the high and low densities of a negative is called *contrast*. The difference in brightness in registration of the highlights and the shadows is the contrast of the film.

Normal contrast would have a range of densities, including highlights, halftones, and shadows. High-contrast film cuts out the halftone range, so that light intensities above a certain value are recorded as bright highlights and all other (lower) intensities are recorded as deep shadows. Such film is normally used for copy work of black-and-white drawings, lettering, and maps. Medium- and low-contrast films record a span of halftones, and are used for most subjects, including microfossils.

The selection of film for photomicrography depends to a great extent on the lighting used. If the subject is illuminated with "soft" lighting (with all sides of the microfossil illuminated and with only slightly greater intensity on the highlighted side), the film should have appreciably higher contrast to give comparable results to those obtained with low-contrast film and with "harsh" lighting (spotlight for the highlighted side and weak lights to fill in the shadow side of the specimen).

The inherent contrast of the emulsion is controlled by its manufacture, but this built-in contrast can be adjusted in the development of the film and in the printing. More will be said about this in the discussion of "Time-gamma." Most films can be developed in several solutions and some cause less contrast than others in the developed image. With a standard developer, the longer the development time, the greater the resulting contrast in the film. Such "playing around" with different developers and/or different development time, however, can only change the contrast by a limited degree from its inherent properties.

In printing, the selection of high-contrast ("hard") paper can strengthen the contrast in the low-contrast negative. Conversely, a low-contrast ("soft") paper can tone down the contrast in a harsh negative. A photographer should be aware of the limits of such choices. Some negatives are too contrasty to print on any commercial paper, and some are too weak to be brought out on available papers. Even "burning in" the highlights by selective overexposure in printing may not be sufficient to show details if the negative is extremely dense on these spots; and even "dodging" the shadow areas by selective underexposure can never show details that are not registered to some degree in the negative. It is far better to achieve a balance of lighting of the specimen and contrast in the film such that any burning or dodging is rarely, if ever, necessary in printing.

RESOLVING POWER. The ability of the emulsion to reproduce fine detail is termed its *resolving power*. This is commonly measured as the number of lines per millimeter which can be recorded clearly discernible in the film. Highspeed films fall in the range of 50 lines or less, and certain slow films will record as many as 250 lines. Persons with normal vision resolve no more than 6 lines per millimeter, so that only where great magnification is necessary in printing the negative does resolving power assume a significant role.

Resolving power is closely related to the grain size of the film; the finer the grain, the greater the resolving power. Very few emulsions are made that deviate much from the 40 to 55 line span, and all are below the resolving power of the average lens.

In considering the resolving power of the lens (apart from the registration on film), calculations can be made for "perfect" lenses. Because of the finite wave length of light (averaging about .0005 mm in the visible spectrum), the image of a narrow line of light formed by a perfect lens consists of a broad line of light bordered on the sides by a series of progressively fainter parallel bands of light and darkness. If two very close narrow parallel bands of light are transmitted by the lens, their images will consist of the summation of light intensities from both. Their identity as two lines can be detected only when the intensity between them is less than **0**.8 of the central intensity of each. This occurs if the two lines of light are separated by at least a distance of 1.12 wave lengths x focal length/ thef-stop of the aperture. In the table below of the resolving powers of the perfect lens, oddly, the larger apertures give theoretical larger numbers of lines per mm than do smaller apertures. However, the actual lenses produced have their greatest aberrations near the edges, and their resolving powers are far below those of the perfect lens. Nevertheless, it could be useful to remember that with some lenses, the resolving power is better at intermediate f-stop openings than at very small openings.

f-stop	1.5	2	2.8	4	5.6	8	11.3	16
lines/mm	1190	890	640	450	320	220	160	110

Associated film properties. -- The properties or characteristics of film are not independent of one another. Films tend to fall into or between two extremes:

	Type	
	I	.II
Speed	Fast	Slow
Grain	Coarse	Fine
Contrast	Low	High
Resolv- ing power	Low	High
Latitude	Wide	Low

Some very specialized films combine fast speed and wide latitude with a significantly finer grain than that found in the usual commercial films of this kind; all require special development.

In optical photomicrography, the desirable fine-grained, high-contrast, high resolving power films usually are accompanied by slow speed and low latitude. The latter characteristics do not affect the operation of a "standard" set-up much; the exposures are normally in seconds, and doubling or even tripling the exposure time does not slow up the procedure unduly; and, once correct exposure is established, wide latitude in the emulsion is unnecessary. Subject brightness range. -- Two factors affect the relative and actual densities of various points in the negative: the subject brightness range and the densities imposed on the film by the development.

If the subject has the brightest spot that is to be recorded with detail only 4 times as bright (as could be measured by accurate lightmeter) as the darkest shadow spot that is to be recorded with detail, the subject brightness range (SBR) is 4, which is very low in contrast (said to be "flat"). For example, a meter reading of the shadow area is 8 and the reading of the highlight is 32, the ratio is 32/8 = 4/1 and the SBR is 4. If the brightest spot is 250 times as bright as the darkest spot, the subject is very contrasty.

From the results obtained in the negative during experimentation, an estimate can be made of the SBR and used in planning improvements to the lighting and exposure.

Negative density range. -- This topic can be discussed here perhaps better than after the section on "Photographic Chemistry," because the evaluation of the negative may lead to alterations in the lighting and exposure time.

The negative density range (NDR) compares how much light gets through the negative at the thin spots (image of the shadow spots in the subject) as compared with how much light gets through the negative at the dense spots (image of the highlights in the subject). The photographic computations involving density are made to conform with the American Standard for Diffuse Transmission Density. The explanation of NDR is not as formidable as it appears. Simply,

 $NDR = Density_{(max)} - Density_{(min)}$ 

In other words, NDR is the difference between the strong transmission through the thin spots compared to the weak transmission through the dense spots in the developed negative.

Using the following symbols:

- Io = light intensity (technically, "luminous flux")
  incident on the negative
- I<sub>t</sub> = light intensity transmitted through the negative

$$\begin{array}{l} T &= \mbox{transmission of the negative} \\ O &= \mbox{opacity of the negative} \\ D &= \mbox{density of the negative} \\ T &= \box{I}_t \\ D &= \mbox{log O} = \mbox{log } \box{I}_t \\ D &= \mbox{log O} = \mbox{log } \box{I}_t \\ \end{array}$$

Thus, density is equivalent to the negative logarithm of the transmission of the negative. It can be measured for the thinnest and densest parts of the negative. A simple way to measure the values of  $I_o$  and  $I_t$  would be to take a spot reading with a light meter of the light coming through an enlarger without the negative in place, and another reading of a projected spot of the image as projected through the negative. Divide the first figure obtained by the second to get the opacity of that spot on the negative; the density is the logarithm of the opacity.

<u>Gamma.</u> -- The development of the negative permits adjustment of the contrast in the subject as registered in the latent image. A numerical measure of the contrast provided by the development process is called *gamma*. Since time, temperature, and agitation, as well as the composition of the developer and the type of film, affect the final contrast in the developed negative, all these factors are embodied in the concept of gamma.

The aim of development control is to achieve a negative that is (1) capable of showing clearly as many details of the subject as are deemed necessary to portray its essential character, and (2) capable of being printed on commercially available printing paper to show such details to advantage.

Gamma expresses a relationship between subject brightness range (SBR) and the negative density range (NDR):

$$Gamma = \frac{NDR}{\log SBR}$$

As shown in the Table of Negative Density Ranges, a negative density range of .90 could be achieved for a subject brightness range of 4 if properly exposed and if the development process could be "pushed" to an extreme gamma of 1.5. Most developers reach only as high as gamma of 1.1 or 1.2.

							Subje	ct Bri	ghtņes	s Rang	e (SBR	)					
		4	6	8	10	12	14	16	18	20	24	<b>2</b> 8	32	36	40	44	
	0.4	.24	.31	.36	.40	.43	.46	.48	.50	.52	.55	.58	.60	.62	.64	.66	0.4
	0.5	.30	.39	.45	.50	.54	.57	.60	.63	.65	.69	.72	.75	.78	.80	.82	0.5
	0.6	.36	.47	.54	.60	.65	.69	.72	.75	.78	.83	.87	.90	.93	.96	.99	0.6
lent	0.7	.42	.54	.63	.70	.76	.80	.84	.88	.91	.97	1.01	1.05	1.09	1.12	1.15	0.7
lopm	0.8	.48	.62	.72	.80	.86	.92	.96	1.00	1.04	1.10	1.16	1.20	1.24	1.28	1.31	0.8
eve.	0.9	.54	.70	.81	.90	.97	1.03	1.08	1.13	1.17	1.24	1.30	1.35	1.40	1.44	1.48	0.9
ЪЪ	1.0	.60	.78	.90	1.00	1.08	1.15	1.20	1.26	1.30	1.38	1.45	1.50	1.56	1.60	1.64	1.0
ma	1.1	.66	.86	.99	1.10	1.19	1.26	1.32	1.38	1.43	1.52	1.60	1.65	1.71	1.76	1.81	1.1
Gam	1.2	.72	.93	1.08	1.20	1.30	1.38	1.44	1.51	1.56	1.66	1.74	1.80	1.87	1.92	1.97	1.2
	1.3	.78	1.01	1.17	1.30	1.40	1.49	1.56	1.63	1.69	1.80	1.88	1.95	2.02	2.08	2.13	1.3
	1.4	.84	1.09	1.26	1.40	1.51	1.61	1.68	1.76	1.82	1.93	2.03	2.10	2.18	2.24	2.30	1.4
	1.5	.90	1.17	1.35	1.50		1.72	1.80	1.88	1.95	2.07	2.17	2.25	2.33	2.40	2.46	1.5
							Subjec	t Brig	htness	s Range	e (SBR)						
		48	52	58	64	70	80	90	100	120	140	160	180	200	220	240	
int	0.4	.67	.69	.71	.72	.74	.76	.78	.80	.83	.86	.88	.90	.92	.94	.95	0.4
opme	0.5	.84	.86	.88	.90	.92	.95	.98	1.00	1.04	1.07	1.10	1.13	1.15	1.17	1.19	0.5
vel	0.6	1.01	1.03	1.06	1.08	1.11	1.14	1.17	1.20	1.25	1.29	1.32	1.35	1.38	1.41	1.43	0.6
De	0.7	1.18	1.20	1.23	1.26	1.29	1.33	1.37	1.40	1.46	1.50	1.54	1.58	1.61	1.64	1.67	0.7
a of	0.8	1.34	1.37	1.41	1.44	1.48	1.52	1.56	1.60	1.66	1.72	1.76	1.80	1.84	1.87	1.90	0.8
amme	0.9	1.51	1.54	1.58	1.62	1.66	1.71	1.76	1.80	1.87							0.9
G	1.0	1.68	1.72	1.76	1.80	1.85											1.0

TABLE OF NEGATIVE DENSITY RANGES

Nevertheless, using a developer which can attain a range of gamma from 0.5 to 1.1, it can be seen in the table that a negative density range of .90 can be reached for a SBR of 64 by developing to gamma = 0.5; or for a SBR of 32 by developing to gamma = 0.6; or for a SBR of 20 by developing to gamma = 0.7; or even for a SBR of about 7 by developing to gamma = 1.1 (very high). Without special papers and developing solutions, a negative density range below.50 or above 1.40 is difficult to print satisfactorily, as indicated by the dotted areas on the table. This table is useful in adjusting development to give the most readily printed negatives from the lighting-lens-aperture-magnificationexposure set-up. Or the SBR can be adjusted by changing lighting so that all development can proceed at a selected gamma.

How gamma is reached in development is discussed under Photographic Chemistry.

### PHOTOGRAPHIC CHEMISTRY

The procedure for making the image visible is the same for film or paper -- passing the material through successively: developer, fixing bath, and wash. The only difference is in the chemicals used, particularly in the developer.

Developer or reducing agent. -- Although many chemicals are capable of reducing silver halides to metallic silver, only a few are selective enough to reduce only the exposed halides. All others are useless, since they reduce all halides in the emulsion and turn it solid black.

Pioneer photographers (before 1851) knew only ferrous oxalate --  $FeC_2O_4 \cdot 2H_2O$ . Development was a messy business, because the solution stained hands and (without special care) the emulsion. Around 1851 it was replaced by pyrogallol, and later, around 1880, by hydroquinone. Metol was discovered in 1891, and modern developers evolved thereafter.

PYROGALLOL. This phenolic compound -- $C_6H_3(OH)_3$  -- was not entirely satisfactory as a replacement for ferrous oxalate. It quickly became oxidized in solution and it also stained everything with which it came in contact: hands, negatives, and equipment. By the amount of alkali added in its preparation, the degree of contrast produced by pyro could be controlled. The stains left on negatives were not regarded by the old-time photographers as a detriment, for it had a softening effect on the resultant prints.

METOL. Also known in the photographic trade as "Elon," "Pictol," or "Rhodol," metol --  $C_{14}H_{18}N_2O_2 \cdot H_2SO_4$  -- is an energetic developer that brings out the image quickly but builds density very slowly (said to be "softworking"). It has the advantages of being fairly independent of temperature and large quantities of restrainer. Because it is difficult to obtain contrast with metol alone, it is usually combined with other developers, such as hydroquinone.

HYDROQUINONE. This developer, which has a composition of  $C_6H_4(OH)_2$ , is low-energy and produces great density in the highlights while retaining transparency in the unexposed areas. It makes a harsh contrast in the negative by building up extreme density in the exposed areas of the negative. It is used alone for high-contrast reproductions of drawings, but for general use it is mixed with metol to subdue its harshness and bring out the halftones.

Hydroquinone is sensitive to temperature; below 50°F it becomes inert and above 80°F it tends to fog. It does keep well in solution and oxidizes slowly in air.

METOL-HYDROQUINONE. Usually referred to as M-Q developer, it is versatile, having the desirable qualities of both metol (bringing out detail) and hydroquinone (building up density and contrast). It keeps well in solution and works faster than either of its ingredients used separately.

AMIDOL. This developing agent, composed of  $C_6H_8N_2O \cdot 2$  HCl, acts very quickly. It is one of a very few developers that can be used without an accelerator or restrainer, but it oxidizes and deteriorates quickly in solution. Amidol and a preservative make a solution that yields satisfactory results for many negatives.

PARAPHENYLENE-DIAMINE. This developer -approximately  $CH_3CONHC_6H_4NH_2$  (N-acetyl-ophenylenediamine, or o-amino-acetanilide) -is often used with a preservative only (sodium sulfite) to give negatives of extremely fine grain where enlargement is planned. Because it is slow acting, it is often combined with "Glycin," which also produces fine grain but does not so readily oxidize.

Accelerator. -- A developing agent alone in the water solution does little or no developing because it is neutral to acid. For the reactions to proceed and for the reducing agent to work, the solution must be alkaline. To bring this to be, an *accelerator* is added. It accomplishes two purposes: it energizes the developing agent to perform its task, and it also softens the gelatin emulsion, permitting faster penetration of the developing solution. The amount of alkali added determines the "energy" of the development. Too little alkali slows the development; too much results in very high contrast and even in chemical fog when the reducing agent is pushed to the point where it starts developing unexposed halides. Concentrated alkalis may swell the gelatin so much that it blisters and "frills" at the edges. High-energy developers, such as metol, require less alkali than the slow lowenergy developers, such as hydroquinone.

Accelerators are divided into three general types according to their strength and effect in development: *strong*, *moderate*, and *mild*.

The strongest accelerators are sodium hydroxide (NaOH) and potassium hydroxide (KOH). These caustic alkalis are used with some developers for high contrast. Their action is so strong that they are excluded from the other developers. They increase grain size in the softened emulsion by causing the silver grains to clump together.

Sodium carbonate  $(Na_2CO_3)$  is a mediumstrength accelerator that is commonly used, as in M-Q developers. Potassium carbonate  $(K_2$  $CO_3)$  could be used as a substitute, but is less stable in solution and more expensive than the sodium carbonate.

The mildest alkali is sodium borate or borax  $(Na_2B_4O_7 \cdot 10 H_2O)$ , often used in the fine-grain developers. It is sometimes called a "buffer" alkali solution, because it slowly and continuously releases its alkali content, keeping alkalinity nearly constant. Sodium metaborate  $(NaBO_2 \cdot 2 H_2O)$  is slightly stronger, but acts in the same manner.

The accelerator influences the graininess produced by development. The more active the accelerator, the more the silver particles tend to clump together, and the larger the resulting grain of the negative.

Restrainer. -- Strangely, developers need a *restrainer* in addition to an accelerator, because without a restrainer the reaction proceeds too rapidly and some of the unexposed halides will also be converted into metallic silver. This causes fogging of the negative. The addition of a restrainer to the solution prolongs the development time and reduces fogging to a minimum.

Potassium bromide (KBr) is most common as a restrainer, but sodium bromide (NaBr) can be substituted. Potassium iodide (KI) could be used, but it is not popular because it takes longer in the fixing bath to be removed.

Preservative. -- Organic developing agents in an alkaline solution have a strong affinity for oxygen; when they are exposed to air they quickly oxidize and lose their photographic capability. To prevent this, a chemical is added -- sodium sulfite  $(Na_2SO_3)$  -- with a greater affinity for oxygen than the reducing agent. Whenever the solution is exposed to air, the sulfite combines with it before the developing agents can react. The *preservative* not only prolongs the useful life of the developer, but it also prevents the formation of unwanted oxidation products, which can cause stains on the negative.

The way a solution is to be used determines the amount of preservative needed. If the solution is to be mixed, used immediately, and then discarded, very little preservative is needed. Solutions used in trays require more sulfite than those used in tanks, because the exposure to air speeds oxidation. Developers kept at high temperatures or much diluted also need more preservative than usual.

Sodium metabisulfite (NaHSO<sub>3</sub>) may also be used as a preservative. Because pyro is more readily oxidized in alkaline solution than any other developer, sodium metabisulfite is often used in pyro developers; it is slightly acid and preserves the solution longer.

Rinse bath. -- To remove surplus developer from its surface, the negative or print is placed in a rinse bath. Three kinds of rinse baths may be used: water, acid, and hardening.

Water simply rinses off the developing solution so that it does not contaminate and counteract the fixing bath.

An acid rinse bath, commonly referred to as the "short stop," is a weak solution of acetic acid (CH<sub>3</sub>COOH). It stops all development by neutralizing the action of the developer, and thereby prolongs the life of the fixing bath.

A hardening solution for the rinse bath, composed of potassium alum  $(K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O)$  is used in tropical laboratories where temperature cannot be controlled. It hardens the emulsion. In most laboratories it is unnecessary and only the acid "short stop" is added to the rinse bath. Fixation. -- Developing the film or paper does not complete the developing process. After the film is developed it still carries the halides where no light struck it, as well as the converted metallic silver where light originally struck it. Before exposure to light, it is necessary to place the film in a *fixing bath*. This solution makes the unexposed and unaffected halides soluble in water, dissolves them from the gelatin, and leaves the image of metallic silver embedded in the gelatin support.

If the film is not completely "fixed," at least some of the unexposed halides remaining in the emulsion will sooner or later upon exposure to air change to metallic silver, spoiling or completely ruining and obliterating the image.

The fixing solution contains a halide solvent, acetic acid, sodium sulfite, potassium alum, and boric acid. Each serves a purpose.

SILVER HALIDE SOLVENT. The most common chemical used to dissolve the unexposed halides is sodium thiosulfate  $(Na_2S_2O_3 \cdot 5H_2O)$ , better known as *hypo*. It was used as a fixing agent as long ago as 1820, and was mistakenly thought to be a different chemical, sodium hyposulfite  $(Na_2S_2O_4 \cdot 2H_2O)$  -- hence the nickname "hypo." It converts the halides into soluble compounds. It would perform its task alone, but other ingredients are added to make the solution last longer and act more efficiently.

ACETIC ACID. The acetic acid in the "short stop" may not be sufficient to neutralize the alkalinity of the developer. If the developer is still alkaline when it contacts the plain hypo, it begins to oxidize and causes stains. Therefore, acetic acid is added to the fixing bath as well as the rinse bath.

SODIUM SULFITE. This chemical, which is added to the developing solution as a preservative, is also added to the fixing solution. The addition of acetic acid (to neutralize the developer) causes the hypo to disintegrate into free sulfur and sulfuric acid. When sodium sulfite is present in the solution, it combines with the sulfur to form new hypo; in counteracting the adverse action of the acetic acid, it prolongs and preserves the fixing solution. POTASSIUM ALUM. This chemical, the same that was added to the hardening rinse bath for tropical darkrooms  $(K_2SO_4 \cdot Al_2(SO_4)_3 24 H_2O)$ is a regular addition to the fixing solution, in which it serves the same general purpose. It toughens and hardens the emulsion, which becomes susceptible to scratches as it softens and swells in the developing process.

BORIC ACID. The potassium alum added to harden the emulsion causes precipitation of aluminum sulfite  $(Al_2(SO_3)_3)$ , a milky sludge, when the solution becomes neutralized. Boric acid slows the precipitation and extends the useful life of the fixer.

The fixing solution, therefore, starts out with hypo -- the basic chemical to do the job -and other chemicals are added to keep the hypo active; they have disadvantages that must be overcome by still other chemicals. The combination that fulfills the function with fewest drawbacks is the solution including the chemicals listed above.

TIME OF FIXATION. Films should be left in the fixer for twice the length of time it takes to clear them. Prints should remain in fresh hypo for at least 10 minutes. When fixation appears to take too long, the hypo is exhausted and unable to perform; it should be promptly discarded and new solution supplied in its place.

Some agitation is needed for films and prints to bring other hypo in contact with the surface. Films or prints stacked on one another in the fixing solution cannot be acted upon; problems of incomplete fixation may not appear for some time afterward (when oxidation begins to produce stains) and new prints or films will have to be exposed and processed.

Washing. -- If hypo is not completely eliminated from the negative or print, it will eventually decompose and attack the image. Washing with generous agitation for at least 20 minutes will wash the last traces of the fixer from the negative; heavy-weight papers may require over an hour to become hypo-free.

Drying. -- Drying of negatives should proceed slowly as they are suspended in a dustfree room; Otherwise, dust particles may be entrapped in the moistened and soft gelatin. Papers are less delicate and can be dried much faster by a heated dryer to give the proper finish (matte or glossy) to the emulsion side.

Water used in making solutions. -- If possible, distilled water should be used in mixing all developing and fixing solutions. The presence of foreign matter, especially coagulants, may spot negatives as it becomes entrapped in the gelatin. Iron in the water is particularly troublesome, for it oxidizes and uses up the sodium sulfite in the developer.

If water is clouded with slime and suspended particles, it may be cleared by adding 15 grains of potassium alum to each gallon of water; the cleared water can be decanted and the settled impurities thrown away. The presence of the alum does not affect either developer or fixer inasmuch as it is a necessary ingredient in both.

#### PRINTING

The basis on which the whole photographic performance is judged is the final print. This is a fair basis, for a poor exposure can never lead to a good negative, a poor negative can never lead to a good print, and even a properly exposed print can be spoiled by poor technique. So, any inefficiency or error in the entire process will show up in the print.

Procedure. -- Printing is done by one of two general procedures: contact and projection.

CONTACT. The photomicrographer is not apt to make negatives the same size as the final print. For one reason, the added depth of field obtained at lower magnification on film is critical to successful definition in the print. Furthermore, if a number of photographs are to be taken, the 35-mm film has a distinct advantage over larger film -- devloping can be delayed until all exposures are made (once the set-up is perfected), saving a great deal of time that would otherwise be consumed in changing film after each exposure. We need not consider contact printing further; if it is needed, most readers will be fully familiar with it anyhow.

**PROJECTION PRINTING.** Using a standard model of enlarger to project the image of the

negative onto the printing paper will eliminate any problems of matching the light source, condenser, and lens in the enlarger to give even illumination of the field.

One of the advantages accrued from enlargement of 35-mm negatives is the control of magnification of the specimen on the print. If, as in most studies, many or all of the photos are to be made at a certain magnification, the camera can be set once to make all negatives to the same enlargement and the enlarger can be set once to bring all negatives to the same projected enlargement.

It is helpful to the reader of the final publication to see the pictures of microfossils at definite magnifications -- all on a plate at x 25, or all at x 40 -- with exceptions only to show "close-up" details. Hence, the magnification in the final printing of the paper must be considered before any print is made. If the plates are to be reproduced at natural size, then the magnification in the print will be the magnification as it will appear in the published plate; but if the plates are to be reduced to 60% of their actual size in publication and it is desired to have the photographs published at x 40, then the plate must be made 100/60 of the anticipated journal size and the magnification of the prints must be  $100/60 \ge 40 = \ge 66.7$ . For judging clarity and contrast, it is a good policy to make prints and plates at publication size.

Another advantage of projection printing over contact printing is the opportunity to correct what may be termed "unavoidable deficiencies" in the negative. Suppose the specimen has fine ornamentation of low relief, which can only be discerned when it is lighted by a strong, lowlevel highlight. Such high-contrast lighting will throw the ornamentation into proper "relief" but may obscure details in some highlighted areas of the specimen and will certainly give insufficient illumination to the shadow areas. Because of the necessity for special lighting to show certain details, a "poor" negative is produced; because the emulsion responds equally over all its surface, and cannot be made selective, the highlighted areas of the specimen will be shown greatly overexposed and overly dense in the negative; on the other hand, the shadow areas of

the specimen will be badly underexposed and thin in the negative. To a limited degree, this harshness can be alleviated in projection printing by "dodging" and "burning in."

To bring out any registration of detail in the thin shadow areas of the negative, the light passing from the film image to the printing paper can be blocked out for part of the exposure time by intercepting the rays with some solid object (a special cut-out of paper, a pencil, a finger, or any object that can be made to fit along the shadow side margin of the image). This is "dodging" of part of the negative. Conversely, to bring out any detail still not blocked completely by the clumps of precipitated silver in the dense highlight areas of the negative, the light passing from the film image to the printing paper can be allowed to fall on this area an extra amount of time by passing the rays from the film image to the printing paper through a hole in an opaque sheet of paper or cardboard. which shields off the remainder of the projected image. This "burning in" brings the tone of the highlighted area closer to the rest of the image. Such manipulations with the projected image must be done with artful care. The object used to dodge or burn should be kept well above the paper (on which the image comes into focus) in order to keep the rays passing the edges of the object diffracted rather than sharp. In addition, the underexposure or overexposure of the areas in printing should be gradational, with the shadow areas progressively underexposed from the undoctored area to the edge of the specimen's image, and the highlights progressively overexposed from the undoctored areas to the center of highlight density.

Sometimes a compromise can be reached between the need to emphasize details of lowrelief structures and the desirability of a balanced negative. It is always true that details that never registered in the shadow areas of the negative and details that are completely blocked out in the highlight areas of the negative can never be made visible in the print. Nevertheless, careful and considerate dodging and burning can extend the tonal range in problem shadow and highlight areas. In any case, the departure from a balanced lighting should be the minimum required to show the features which would otherwise be too subdued to discern.

Types of paper. -- Printing papers are manufactured in several inherent contrasts, several finishes, and several weights.

CONTRASTS. Paper is made in numerous inherent contrasts, of which numbers 1 through 5 are widely available. Number 1 is a "soft" paper, reserved for printing very contrasty negatives; number 5 is a so-called "hard" paper, designed to strengthen the contrast in very flat negatives. The "average" negatives should print on number 2 or 3 paper.

FINISHES. Omitting the "special effects" finishes and textures, which are unsuitable for scientific work, the choice narrows to two common finishes -- glossy and matte. For prints that must be blackened around the edges of the picture to blend into a black background, matte finish will hold the blacking compound better than glossy, which tends to flake off with handling. Many photographers prefer glossy, however, because the image appears brighter.

WEIGHTS. In weight or thickness, printing papers may be half-weight, normalweight, or double-weight. Half-weight papers are very thin and crease easily. Although they are suitable for scientific illustration, they are seldom used because they are somewhat fragile and must be handled with more care than thicker papers. Double-weight papers resemble postal cards in thickness, and are mostly used for commercial portraits and exhibition prints. Normal-weight or "single-weight" paper is most readily available and fulfills the requirements of durability, strength, and easy processing.

#### PROCESSING ROUTINE

Precautions.-- In processing either film or prints, certain precautions are worth noting.

CLEANLINESS. Particularly films are liable to damage and ruin by sloppy techniques. Stains spoil the image for printing and dirt can scratch the emulsion or become embedded in it.

Even if a dust-free air circulation is not available, frequent cleaning and scrubbing of all floors, walls, and equipment in the darkroom will sooner or later pay dividends in avoiding negative and print damage. Exposing a few new negatives per day, after all camera equipment has been dissembled and stored, can involve much more time than keeping the darkroom clean.

TEMPERATURE CONTROL. Developer, rinse bath, and fixing solution should be at nearly the same temperature. Passage from cold to hot solutions and the reverse can cause strains in the emulsion which may exceed its elastic limit. Sudden chilling and contraction may cause the emulsion to crack, and sudden heating and expansion may cause wrinkling or reticulation. All processing solutions can be brought to about room temperature before using by placing the trays containing the solutions in a water bath.

MAINTAINING SOLUTION PURITY. Splashing the solutions as the print or film is passed into them can have dire results. The developer can be rendered useless by a few drops of the short stop bath. The hypo can be exhausted by dumping a number of unrinsed prints directly into it. Contamination of solutions is to be avoided.

Darkroom layout. -- Within the same space a convenient or very inconvenient layout can be arranged. If possible, the processing of film should be separated from the processing of the prints.

The lab space for film processing can include the stored film (in bulk, if cartridges are to be loaded), work table for developing, and lines for drying films. The lab space for paper processing can include the enlarger, storage of various grades of paper, work table for processing, and drying facilities.

Proper selections of safelights should be installed in each room, together with normal lights for inspection of results and for routine cleaning and other operations. The chemicals used in making up the solutions, or the made-up solutions, can be stored if necessary outside the darkroom but close at hand. Cameras, lenses, and lights for shooting should never be stored in the darkroom where they are apt to be affected by chemicals used in processing.

Proper ventilation of the darkroom is a must. Fumes should be promptly removed by forced circulation of the air. Bulk photography. -- Numerous photomicrography jobs will entail production of many photographs at the same magnification. Speed in production and uniformity in results can be expedited by the establishment of a routine.

MAGNIFICATION. Adjust the camera to give a desired magnification on the film. If the camera is unadaptable and lacks a bellows for bringing a specific magnification to the film, set the camera to give maximum enlargement of the largest specimen in the group to be photographed; then photograph a millimeter scale (divided into hundredths, if such is available) on one frame of the roll. Maintain this setting throughout the photographic project. Such planning is better than trying to establish the magnification of each specimen later by comparing actual measurements against the print.

LIGHTING AND NEGATIVE CONTRAST. Allow a few short sections of film for determining the best lighting of the specimen to show its essential characteristics, the best diaphragm setting to get sufficient depth of field, the best exposure time to give a negative of good balance, and the best developer to yield good contrast and density. Inspection of the first developed frames may point up the need for modification of the set-up. When "perfected," make a record of all factors for future reference and use. There is no need to repeat this phase of experimentation for each photographic job.

Proceed to shoot all the specimens which can be advantageously illustrated with this setup. Postpone any special lighting or higher magnifications that may be indicated or desired. Insofar as possible, treat each class of special problems in a new routine. Again, record the factors responsible for giving the best results.

PROCESSING FILM. Even though several hundred frames of film are to be processed at one time, show equal care in handling each roll. Keep developing temperature constant. Be especially careful not to scratch undried film.

Note any deficient negatives and re-shoot before modifying the set-up.

PRINTING. Adjust the enlarger to give the desired final enlargement on the printing paper, using the frame bearing the image of the milli-

meter scale. Select an average negative and find the ideal lens opening and exposure time for the enlarger to give a print which will develop in the recommended time for the paper-developer combination (usually  $1\frac{1}{2}$  to 2 minutes). Expose the first print of each frame for this time, and modify the time to give a print that will match the other pictures in contrast and density closely. Seldom will the first print be exactly right. Nevertheless, the production-line approach to printing of the production-line negatives is a time-saver.

#### **REVISING AND IMPROVING THE NEGATIVE**

Factors in processing an emulsion. -- It is true that many factors affect processing: the type of emulsion, the chemical composition of the developing solution, time, temperature, amount of dilution, and kind of agitation. The emulsion has already been selected by this stage, and the film manufacturers offer certain recommendations for the choice of developer. We can assume that such a choice has been decided before turning to the other factors.

TIME. The length of processing time can be used to control the density and contrast of the exposed negative, within limits. As will be expressed in the section on "Negative Faults," however, no developing process can bring out details in shadow areas if the rays from the subject did not result in any change in the halides in that part of the negative; and no process can bring out details in highlight areas if the light from the subject was so strong that the development changes all the silver halides to metallic silver throughout the area of the negative. Nevertheless, prolonged development can make an acceptable negative from what would be otherwise too flat; and shortened development can make a good, printable negative from one that would otherwise be over-contrasty.

Ideally, the time can be set within narrow limits of the recommended time by changing the exposure of the negative or altering the lighting of the subject.

TEMPERATURE. One of the most often ignored and yet one of the most influential factors in development is the temperature of the solution. In one film and developer, for example, the same contrast is produced by processing for 2 minutes at 90°F as would be produced in the same film (exposed the same time) and developer by processing for 10 minutes at 55°F. Of course, these extremes of temperature are not recommended, and a preferable development would be about 7 minutes at 68°F.

The manufacturer's recommendations for each film should be heeded. Some are unduly influenced by high temperatures and react with a variety of damaging and blemishing maladies. Most films are designed for best results when developed at 68°F.

AGITATION. The movement of the solution over the emulsion promotes devlopment as it brings a fresh supply of the chemicals into contact with the surface. For that reason, films and/or papers processed in a tray with agitation develop faster than films dipped into a tank or allowed to rest in a tray. The effects of agitation are very secondary to those produced by additional time or higher temperature.

TIME-GAMMA-TEMPERATURE CHARTS. For films in particular, these charts supply the photographer with information to plan his processing and to improve his results.

Gamma has already been discussed. It is a factor relating the density range in a correctly exposed negative (NDR) to the brightness range in the subject (SBR)

$$Gamma = \frac{NDR}{\log SBR}$$

The gamma achieved in development can bring out the full potential of the latent image in the film and make it printable.

With each commercial developer, the maker supplies time-gamma-temperature information in the form of a chart for each of the films for which the developer is likely to be used. The time-gamma-temperature chart for the particular film-developer combination is a graph with temperature in °F or °C as the ordinate and time of development in minutes as the abscissa; sloping subparallel lines across the graph are labeled with values of gamma produced by those temperatures and times.

As shown in the following table of gamma adapted from the data of a typical time-gamma-

															فليشبعهن
	Values of Gamma														
		Developing Time (minutes)													
		2	3	4	5	6	7	8	9	10	12	15	20	25	30
	90°	.52	.67	.80	.91	1.01	1.09	1.15							
~	85°		.49	.59	.70	.79	.89	.96	1.01	1.07					
.н.)	80°			.48	.56	.63	.72	.79	.88	.93	1.02	1.11			
ıre	75°				.47	.54	.59	.67	.74	.79	.90	1.02			
ratı	70°					.48	.54	.57	.62	.69	.80	.93	1.07		
adua	65°						.48	.53	.57	.60	.70	.83	1.00	1.10	
Ē	60°							.49	.53	.56	.61	.74	.91	1.03	1.12
	55°								.49	.53	.58	.67	.82	.96	1.06

DATA FROM TYPICAL TIME-GAMMA-TEMPERATURE CHART

Values of Developing Time (minutes)

					Gamma	ι		
		0.5	0.6	0.7	0.8	0.9	1.0	1.1
	90°	1.8	2.5	3.2	4.0	4.9	5.9	7.1
~	85°	3.0	4.0	5.0	6.1	7.1	8.9	11.3
ч°)	80°	4.2	5.5	6.8	8.1	9.4	11.5	14.8
ure	75°	5.4	7.1	8.4	10.1	12.0	14.3	17.8
erat	70°	6.3	8.6	10.1	12.0	14.4	17.0	21.0
empe	65°	7.3	10.0	12.0	14.3	17.0	20.0	25.0
Ē	60°	8.2	11.5	14.1	16.8	19.7	23.6	29.0
	55°	9.2	12.3	16.0	19.3	23.0	27.0	34.0

Typical gamma values for grades of paper:

Gamma	Grade of contrast
1.10-1.35	No. l
.90-1.10	No. 2
.7090	No. 3
.5570	No. 4
.4055	No. 5
	ANNAN MANANAN MANANANANANANANANANANANANA

temperature chart, the same gamma is reached at high temperature for a short time as at low temperature for a long developing time.

After a few experiments, the photographer can decide approximately the value of his

subject brightness range (SBR). Next, he can select the negative density range (NDR) which would fit the printing paper he plans to use, and from the Table of Negative Density Ranges presented above learn what gamma of development will give this NDR. For example, from a few experimental exposures, he finds that his SBR is about 24. To print his negatives on Number 3 paper he desires a NDR of .80; the gamma of development should be about 0.6. Now, if the time-gamma-temperature data given here applies to his film type and developer, he should process his films for 10 minutes at 65°F; this will produce the gamma of 0.6.

By means of gamma, the charts for the film-developer combination in use and the Table of Negative Density Ranges provide interrelations on the lighting of the subject, the development time, and temperature. They act as a guide in the search for the best negative that can be produced with available equipment.

Proper exposure. -- The data on gamma is based on the assumption of proper exposure. The proper exposure time can be derived in two ways.

EXPERIMENTAL. The approach most widely employed in arriving at correct exposure for the particular lighting and lens setting is to expose a number of frames at variant times, develop all at an intermediate gamma, and select the exposure time which produced the best negative density range.

LIGHT METER. If the photomicrographer is fortunate enough to have access to an accurate

through-the-lens light meter, an immediate decision can be made on the proper exposure. With microfossils, which cover only part of the field of the film, however, the background is also registered on the meter. If the background is appreciably darker or lighter than the microfossil, it is better to take the meter reading on a light-gray card which closely matches the overall tone of the specimen as coated.

Negative faults. -- The contrast in the film can be corrected by changing (1) the subject lighting, (2) the development gamma, or (3) both. The lack of detail in the shadow or highlight areas of the image can be corrected by changing (1) the lighting of the subject to make it more nearly uniform and balanced, or (2) the exposure time. Printing on various grades of paper can remedy contrast to a degree, but the negatives produced should rarely if ever require the hardest or softest contrast in printing paper.

The following chart is useful in analyzing the deficiencies of negatives. It serves best in the experimental stage, as various combinations are being tried to discover the ideal one for the series of photographs. It is always better to correct the exposure, the lighting, or the processing than to try to remedy incorrect negatives in printing. This is especially true when numerous photographs are planned for a "standard" set-up.

	Fault	Cause	Prevention	Remedy
ast	Contrasty, lacking shadow detail	Forcing development of underexposed negative	More exposure, less development; more even lighting of specimen at higher level	Soft paper for excess- ive contrast; no rem- edy for lack of shad- ow detail
rrect Contra	Contrasty, full shad- ow detail	Overdevelopment of normally exposed nega- tive	Develop correct time and temperature; use lower gamma; higher angle lighting may help	Soft paper
Inco	Contrasty, highlights blocked	Overexposure, overdev- elopment	Less exposure, shorter development; weaker highlighting of speci- men at higher angle	Soft paper for excess- ive contrast; no rem- edy for lack of high- light detail

TABLE OF NEGATIVE FAULTS

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## TABLE OF NEGATIVE FAULTS (continued)

	Fault	Cause	Prevention	Remedy
rast	Flat, lacking shadow detail	Underexposure, under- development	More exposure, incre- ased development	Hard paper for excess- ive flatness; no rem- edy for lack of shadow detail
rect Cont	Flat, full detail	Underdevelopment of correctly exposed negative	Use higher gamma in development; lower angle lighting may help	Hard paper
Inco	Flat, highlights block- ed out	Development cut too short trying to cor- rect overexposed nega- tive	Expose less, develop normally	Hard paper for excess- ive flatness; no rem- edy for lack of high- light detail
	Dense, highlights blocked, other tones flat	Overexposure and un- derdevelopment trying to correct	Less exposure, full development	Hard paper for flat- ness; no remedy for lack of highlight de- tail
Density	Dense, highlights blocked, other tones correct	Overexposure, correct development	Decrease exposure	No remedy for lack of highlight detail
	Dense, highlights blocked, other tones too contrasty	Overexposure, over- development	Less exposure, less development	No remedy for lack of highlight detail; soft paper for excessive contrast
Incorrect	Thin, lacking shadow detail, other tones flat	Underexposure, under- development	Increase both exposure and development	Hard paper for excess- ive flatness; no rem- edy for lack of shad- ow detail
	Thin, lacking shadow detail, other tones correct	Underexposure, correct development	Increase exposure; softer lighting at higher level of speci- men may help fill in shadows	Normal paper; no rem- edy for lack of shad- ow detail
	Thin, lacking shadow detail, other tones too contrasty	Underexposure, over- development	Increase exposure, de- crease development; softer lighting at higher level of speci- men	Soft paper for excess- ive contrast; no rem- edy for lack of shad- ow detail

#### MULTIPLE LENSES

Very few lenses are now made which are molded equally biconvex of one kind of glass. What may appear as a "single" lens may be three or four lenses mounted in series or cemented together, and ordinarily two or more glass compositions are used in their manufacture. The optical center of such lens series can lie somewhere within the innermost lens or anywhere between the outermost and innermost lens elements.

All of the discussion of photography above applies to what is commonly known as a "singlelens" camera (even though "single unit of lenses mounted together" or "single set of lenses" might be more appropriate.

Experiments have shown years ago that the problem of depth of field -- which concerns

the photomicrographer in particular -- may be improved by using a far lens in the camera system of fairly long focal length stopped down (to give greater depth of field) and a near lens to magnify the image transmitted by the far lens onto the film.

Here a few of the factors that affect a two- or three-lens camera will be discussed. particularly as they apply to the use of a microscope as the lens-bearing part of the camera. As with the composition of the fixing solution, a new ingredient (in this case, lens) is added to counteract a deficiency in the first, and itself introduces new deficiencies which must be counteracted or neutralized by still other additions. The addition of lenses to the camera does not lead necessarily to "perfect" pictures, with "perfect" resolution and "perfect" depth of field; instead, it is the best available compromise between conflicting contributions to better images on the film.

Light transmission of lens. -- Of the light passing through a single lens or a series of lenses, part will be refracted rays from the subject being passed on to form the image on the focal plane and part will be reflected, reflected and refracted, reflected and refracted, etc. rays originating from reflections of the original rays on the glass surfaces. The first rays are the useful light transmitted by the lens to the image of the subject, and the second rays are stray light that has reflected at least twice on lens surfaces before reaching the film, and there tending to destroy the image. The more lenses, the more reflections in the system. To a degree, the glass of which the lens is made controls the division of the light rays into true-



Some reflections set up on three glassair surfaces by an incoming ray.

image rays and unwanted reflections. The per cent of reflected light is higher for glass with a higher index of refraction.

Stray light in lens system. -- The light reflected from a lens surface creates two disadvantages: (1) the intensity of the light transmitted to form the image is diminished by this amount, and (2) the reflected light may, by additional reflections and refractions, reach the film and harm the image. The number of glassair surfaces is an exponent of the useful transmitted light, so that each additional lens reduces the transmission more than did the previous lens. This relationship could be anticipated, since each additional lens starts new reflections from the rays which have already been weakened by reflections on and in the first lens.

### 

#### USEFUL LIGHT TRANSMISSION

Let n = refractive index of glass in lensTOT = total light transmitted through the lens (TRL + RFL)

- TRL = useful ("true image") transmitted light
- RFL = reflected light (per cent)
  - p = number of glass/air or air/glass surfaces in the system

[Equation #31]

 $RFL = \left(\frac{n-1}{n+1}\right)^2$  If n = 1.5, RFL = 0.040 and if n = 1.7, RFL = 0.067; the

mean *RFL* for a glass/air surface = 0.05. *TRL* for one glass/air or air/glass surface = 0.95.

$$TRL = (1 - RFL)^{p} = (0.95)^{p}$$
 [Eq. #32]

No lens transmits 100% of the incoming (incident) light. Each additional lens in a system cuts transmission still more. With seven lenses, less than half of the light is transmitted.

Lenses	p (surfaces)	TOT	TRL	Stray	Stray TRL
1	2	90.48	90.25	0.23	0.24
2	4	82.61	81.45	1.16	1.42
3	6	76.00	73.50	2.50	3.40
4	8	70.37	66.34	4.03	6.08
5	10	65.52	59.87	5.65	9.44
6	12	61.29	54.03	7.26	13.45
7	14	57.58	48.76	8.82	18.09
8	16	54.29	44.01	10.28	23.36
9	18	51.35	39.72	11.63	29.28
10	20	48.72	35.84	12.88	35.94

STRAY LIGHT

$$TOT = \frac{1 - RFL}{1 + RFL (p - 1)}$$
 [Equation #33]  

$$TRL = (1 - RFL)^{p}$$
  

$$Stray = \frac{1 - RFL}{1 + RFL (p - 1)} - (1 - RFL)^{p}$$
 [Eq #34]

Stray light becomes a serious problem when numerous lenses are in a system. With as many as ten lenses, the stray light transmitted by reflections, refracted reflections, reflected reflections, etc., can be as strong as 36% of the "true image" light transmitted.

Illumination of field of image. -- The intensity of illumination on the film is always less than the light intensity falling on the lens, because the glass comprising the lens loses a portion of the incoming light by reflections. In addition, as noted previously, the aperture, which controls the area of the lens through which light can penetrate, has a direct effect on the illumination of the film.

As shown below, the illumination on the film is directly proportional to the transmission of the lens (which varies only slightly from lens to lens) and to the square of the sine of the angle formed at the film surface between the optical axis and a line through the outer limit of the cone of light emerging from the lens. The angle of this emergent ray is independent of the size of the lens or the angle at which the rays entered the lens.

Illumination varies over the field of the image regardless of the overall illumination, being concentrated at the optic axis and decreasing toward the edges of the field. If the angle formed from the diaphragm center to the corner of the film is very large, the difference between illumination at the center of the film and illumination at the corners of the field may be so great that every image appears to be centrally bright and peripherally dark. Obviously, the longer focal length lens and the longer camera will alleviate a serious imbalance in illumination of the field. ILLUMINATION ON THE FILM

- Let  $\theta$  = angle between optical axis and emergent ray from the lens when focused
  - B = brightness of subject
  - $E_{o}$  = illumination on film
    - k = transmission of lens (ratio of light getting through the lens to the light falling on the lens

$$E_{o} = k\pi B \sin^{2} \theta$$

[Equation #35]



Without the second s		
Angle $ heta$	sin <sup>2</sup> 0	Illumination *
0°	.0000	.0000 B
5°	.0076	.0227 B
10°	.0302	.0900 B
15°	.0670	.1999 <i>B</i>
20 <sup>0</sup>	.1170	.3491 <i>B</i>
25°	.1786	.5331 <i>B</i>
30 <sup>0</sup>	.2500	.7461 <i>B</i>
35 <sup>0</sup>	.3290	.9819 <i>B</i>
40 <sup>°</sup>	.4132	1.2331 B
45°	.5000	1.4923 <i>B</i>

\* Illumination in foot-candles, where B is measured in candles/sq.ft.

- Only the angle of the emergent ray is a factor in film illumination; the angle of the entering ray is not a factor.
- The illumination on the film is the same for each f-stop, regardless of the distance of the subject: if the subject is only half as far away, its brightness would be 4 times as great, but its image would be magnified 2 diameters and the light would be spread over 4 times the former area; hence, the illumination per unit area is constant.
- No matter how many lenses are used in the camera system, the emergent ray from each lens controls the illumination passed on to the next

lens, and so on through each of the other lenses until the film is reached.

- VARIATION OF ILLUMINATION OVER THE FIELD OF THE IMAGE
  - Let  $\emptyset$  = angle between line through the center of the aperture and a line from the center of the aperture to any point on the film
    - E = illumination at angle  $\phi$  from the center of the aperture
    - $E_0$  = illumination at center of field
    - $E = E_0 \cos^4 \phi$



Table of Variation of Illumination over Field of Image

Angle Ø from optical axis	Relative illumination (light intensity)
0°	1.000
5°	0.984
10°	0.941
15°	0.870
20°	0.780
25°	0.675
40°	0.344
60°	0.062

Only the central part of the cone of emergent light from each lens in the system transmits fairly even illumination over the field of the image.

4444444444444444444444444444<del>4</del>44

Reversible lenses. -- Some lenses are now on the market which give one set of magnifications when mounted normally and a different set of magnifications when reversed (turned around, mounted "backwards"). The explanation of this "magic" lies in a simple principle of refraction between two lenses. If the lens system contains within it a planoconvex lens and a planoconcave lens with the same curvature, a light beam of parallel rays entering the plane side of the planoconvex lens will enter the lens without change and be condensed by refraction as it leaves the lens. By the time the beam reaches the concave side of the planoconcave lens it may cover an appreciably smaller circle. Then, upon entering the second lens, the rays will be refracted back to their original direction and continue on to the next lens in the system.



If such a lens is reversed, the light beam entering the plane surface of the planoconcave lens will be expanded as it leaves the lens, with each ray diverted away from the optic axis. Those rays reaching the convex surface of the planoconvex lens will again be made parallel. The beam through the small central area of the first lens can thus be expanded to cover the whole area of the second lens.



Of course, the actual optics are more complex than the simplified example given here, but the principles are the same. The difficulties of matching the optical properties of one lens against the other to have good resolution in both directions are so great that such lenses not made by many of the world's leading lens manufacturers.

Two-lens system. -- The addition of a second lens to the optical system of the camera may produce smaller, greater, or the same magnification; it may make the focal length longer or shorter, or leave it the same; it may improve the quality of the image or it may negate the ability of the lens system to form any

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image at all.

The optics of the two-lens system is primarily concerned with changing the focal length. Addition of the second lens does not necessarily enlarge the image produced by the first.

TWO-LENS SYSTEM FOCAL LENGTHS

Let  $f_1$  = focal length of first lens  $f_2$  = focal length of second lens  $f_{(1+2)}$  = focal length of combined lenses q = distance between optical centers of the two lenses

$$\begin{aligned} \frac{1}{f_{(1+2)}} &= \frac{1}{f_1} + \frac{1}{f_2} - \frac{q}{f_1 f_2} \\ \frac{1}{f_{(1+2)}} &= \frac{f_1 + f_2 - q}{f_1 f_2} \\ f_{(1+2)} &= \frac{f_1 f_2}{f_1 + f_2 - q} \\ f_{(1+2)} &= \frac{f_1 f_2}{f_1 + f_2 - q} \\ f_1 &+ f_2 - q = \frac{f_1 f_2}{f_{(1+2)}} \\ q &= f_1 + f_2 - \frac{f_1 f_2}{f_{(1+2)}} \\ f_2 &= \frac{f_{(1+2)}(f_1 - q)}{f_1 - f_{(1+2)}} \end{aligned} \qquad [Equation \#39]$$

Example 1: Two lenses of short focal lengths -f = 16 mm and f = 32 mm -- are separated by 4 mm; what is their combined focal length?

$$f = \frac{16 \times 32}{16 + 32 - 4} = \frac{16 \times 32}{44} = 11.6 \text{ mm}$$

Example 2: If the same two lenses are separated by 44 mm, what is their combined focal length?

$$f = \frac{16 \times 32}{16 + 32 - 44} = \frac{16 \times 32}{4} = 128 \text{ mm}$$

Example 3: If the same two lenses are separated by 16 mm (the focal length of one of the two lenses), what is the combined focal length?

 $f = \frac{16 \times 32}{16 + 32 - 16} = 16 \text{ mm (the focal length of the lens which equalled the separation distance)}$ 

Example 4: If the same two lenses are separated by 48 mm (the sum of the two focal lengths of the lenses), what is the combined focal length?

$$f = \frac{16 \times 32}{16 + 32 - 48} = \frac{16 \times 32}{0} = \text{ infinity!}$$

(See the illustration at the top of the next column.)



The equations above apply also to combinations of positive (convex) and negative (concave) lenses:



Example 5: Two lenses, one positive (convex) and one negative (concave), are separated by a short distance:

$$f_{2}^{1} = -16 \text{ mm}$$

$$f_{2}^{2} = -16 \text{ mm}$$

$$q = 4 \text{ mm}$$

$$f = \frac{-16 \times 32}{32 - 16 - 4} = \frac{-(16 \times 32)}{12} = -42.7 \text{ mm}. \text{ The}$$

$$\text{lens system}$$
is negative, acting as a concave lens and never focusing.

Example 6: A positive lens (f = 16 mm) and a negative lens (f = -32 mm) are separated by 4

 $f_{1} = 32 \text{ mm}$ 

Example 7: The same two lenses (example 6) are separated by 44 mm:

$$f = \frac{-32 \times 16}{16 - 32 - 44} = \frac{-(32 \times 16)}{-20} = 8.5 \text{ mm}$$

Example 8: It is desired to produce a focal length of 100 mm by adding a second lens at 50 mm from a lens of 25 mm focal length. From equation #40:

$$f_2 = \frac{100 (25 - 50)}{25 - 100} = 33.3 \text{ mm}$$

Example 9: Two lenses, focal lengths of 25 and 50 mm, are to be combined to produce a lens system with a focal length of 500 mm; how far apart should they be placed?

40

$$q = 25 + 50 - \frac{25 \times 50}{500} = 75 - 2.5 = 72.5 \text{ mm}$$

Example 10: A camera has a lens system with focal length of 16 mm; what lens should be added 32 mm from it to change the focal length to 10 mm?

$$f_2 = \frac{10(16-32)}{16-10} = \frac{-160}{6} = -26.7 \text{ mm}$$

Example 11: A lens of focal length = 16 mm is combined with one of focal length = -8 mm from which it is separated by 4 mm; what is the combined focal length?

$$f = \frac{-8 \times 16}{16 - 8 - 4} = \frac{-8 \times 16}{4} = -32$$
 mm. The combin-  
ation acts as a

concave lens and does not reach focus.

Example 12: The two lenses of example 11 are separated by 32 mm; what is the combined focal length?

$$f = \frac{-8 \times 16}{16 - 8 - 32} = \frac{-8 \times 16}{-24} = 5.3 \text{ mm}$$

Two-lens System Summary

$f_1$	$f_{2}$	q	f <sub>(1+2)</sub>
+	+	0	$f_1 f_2 / \overline{f_1 + f_2}$
+	+	Short	Short
+	+	$f_{1}$	${f}_1$
+	+	$f_2$	$f_2$
+	+	Long	Long
+	+	$= f_1 + f_2$	Infinity
+	+	$> f_1 + f_2$	No focus
+	-	0	$f_1 f_2 / \overline{f_1 + f_2}$
.+	$< q - f_1$		Focus
+	$= q - f_1$		Infinity
+	$> q - f_1$		No focus
+	$= \neg f_1$	Any	Focus
+	> -f <sub>1</sub>	$f_1 + f_2$	Infinity
+	> -f <sub>1</sub>	$< f_1 + f_2$	No focus
+	$> -f_1$	$> f_1 + f_2$	Focus
+	$< -f_1$	Any	Focus
+	-	Short	Long
+	-	$f_1$	$f_{1}$
+	-	$f_2$	Impossible
+	-	Long	Short

- A supplementary lens attached very close to the lens of a camera focused on subjects at infinity will change the focus of the camera to subjects in a plane whose distance from the camera lens is equal to the focal length of the supplementary lens.
- For two positive (convex) lenses, increasing the distance between them lengthens the combined focal length.
- The limiting case for two positive lenses is reached when the distance between them equals the sum of their focal lengths; at that point, their power becomes zero and the focal length is infinite. This is known as an afocal system and is used in telescopes.
- For a positive (convex) and a negative (concave) lens, increasing the distance between them shortens the combined focal length.
- For a positive and a negative lens, the combination will always achieve focus (be positive) if the numerical value of the negative focal. length is equal or more than the numerical value of the positive focal length.
- For a positive and a negative lens, the combination will never focus (will always be negative and equivalent to a concave lens) if the numerical value of the positive focal length equals or exceeds the sum of the numerical value of the negative focal length plus the distance between the lenses  $-f_1 \ge (f_2 + q)$ . This situation may be described as one in which the lenses are separated by a distance equal to or less than the sum of the focal lengths  $-q \le f_1 + f_2$ .

Three-lens system. -- Because in such a system the innermost lens can only modify the image as it has been transmitted by the two outer lenses, the equations for factors in a three-lens system are derived by considering first the focal length of the combined two outer lenses and then the focal length of this two-lens system combined with the innermost lens. It soon becomes apparent that the direction of mounting the lens system can change the combined focal length of the three lenses. In the equations and examples below, "normal" will be regarded as the position with lens #1 outermost and "reversed" as the position with lens #3 outermost.

For systems having more than three lens-

es, formulas become even longer and more involved. However, the combined focal length of a system with any number of lenses can be solved by first computing the focal length of lenses #1 and #2, then computing for the #1 + #2combination and lens #3, then computing for the #1 + #2 + #3 combination and lens #4, etc., until all lenses have been used in the computation. It is well to remember, in actual practice of computing focal lengths, that the optical center of a lens may not be the geometric center. The main use of such computations in planning for a photomicrographic camera is to determine (1) whether the system will focus at all, and (2) the approximate magnification on film with the available bellows extension.

$$\begin{aligned} f_{(1+2+3)} &= \frac{f_{1}(f_{2}+f_{3}) + f_{3}(f_{2}-q_{(1+2)})}{f_{1}f_{2}f_{3}-f_{(1+2+3)}(f_{2}-q_{(1+2)})} & [Equation #41] \\ &= \frac{f_{1}(f_{2}+f_{3}) + f_{3}(f_{2}-q_{(1+2)}) + f_{1}(f_{2}-q_{(1+2)})}{f_{1}f_{2}f_{3}-f_{(1+2+3)}(f_{1}+f_{2}-q_{(1+2)})} & [Equation #42] \\ &= \frac{f_{1}(f_{2}+f_{3}) + f_{3}(f_{2}-q_{(1+2)}) - g_{2}(f_{3})}{f_{1}(f_{2}+f_{3}) - f_{3}} & [Equation #42] \\ &= \frac{f_{1}(f_{2}+f_{3}) + f_{3}(f_{2}-q_{(1+2)}) - g_{2}(f_{3})}{f_{1}(f_{2}+f_{3}) - f_{3}} & [Equation #44] \\ &= \frac{f_{1}(f_{2}+f_{3}) + f_{3}(f_{2}-q_{(1+2)}) - g_{1}(f_{2}+f_{3})}{f_{1}(f_{2}+f_{3}) - f_{1}(f_{2}-g_{(1+2)}) - g_{1}(f_{2}+f_{3}) - f_{3}} & [Equation #44] \\ &= \frac{f_{1}(f_{2}+f_{3}) + f_{3}(f_{2}-g_{(1+2)}) - f_{1}f_{2}f_{3}/f_{1}(f_{2}+f_{3})}{f_{1}(f_{2}+f_{3}) - f_{1}(f_{2}-g_{(1+2)}) - f_{1}f_{2}f_{3}/f_{1}(f_{2}+f_{3})} & [Equation #44] \\ &= \frac{f_{1}(f_{2}+f_{3}) + f_{3}(f_{2}-g_{(1+2)}) - f_{1}f_{2}f_{3}/f_{1}(f_{2}+f_{3})}{f_{1}(f_{2}+f_{3}) - f_{1}(f_{2}-g_{(2+3)})} & [Equation #44] \\ &= \frac{f_{1}(f_{2}+f_{3}) + f_{3}(f_{2}-g_{(1+2)}) - f_{1}f_{2}f_{3}/f_{1}(f_{2}+f_{3})}{f_{1}(f_{2}+f_{3}) - f_{3}} & [Equation #44] \\ &= \frac{f_{1}(f_{2}+f_{3}) + f_{3}(f_{2}-g_{(1+2)}) - f_{1}f_{2}f_{3}/f_{1}(f_{2}+f_{3})}{f_{1}(f_{2}+f_{3}) - f_{3}} & [Equation #46] \\ &= \frac{f_{1}(f_{2}+f_{3}) - f_{2}(f_{2}+f_{3}) - f_{3}}{f_{1}(f_{2}+f_{3}) - f_{3}} & [Equation #46] \\ &= \frac{f_{1}(f_{2}+f_{3}) - f_{2}(f_{2}-g_{(2+3)}) - g_{1}(f_{2}+f_{3}-g_{(2+3)})}{f_{3}(f_{1}+f_{2}) - f_{1}(f_{2}-g_{(2+3)}) - g_{1}(f_{2}+f_{3}-g_{(2+3)})} & [Equation #46] \\ &= \frac{f_{1}(f_{2}+f_{3}) - f_{2}(f_{2}-g_{(2+3)}) - g_{1}(f_{2}+f_{3}-g_{(2+3)})}{f_{3}(f_{1}+f_{2}) - f_{1}(f_{2}-g_{(2+3)}) - g_{1}(f_{2}+f_{3}-g_{(2+3)})} & [Equation #46] \\ &= \frac{f_{1}(f_{2}+f_{3}) - f_{2}(f_{2}+f_{3}-g_{(2+3)})}{f_{3}(f_{1}-f_{2}-g_{(2+3)}) - g_{1}(f_{2}+f_{3}-g_{(2+3)})} & [Equation #46] \\ &= \frac{f_{1}(f_{2}+f_{3}) - f_{2}(f_{2}-g_{(2+3)}) - g_{1}(f_{2}+f_{3}-g_{(2+3)})}{f_{3}(f_{2}-f_{1}+f_{2}+g_{1}(f_{2}+f_{3}-g_{(2+3)})} & [Equation #47] \\ &= \frac{f_{1}(f_{2}+f_{3}) - f_{2}$$

$$f_1 = \frac{f_2 f_3 - f_{(1+2+3)} (f_2 + f_3 - q_{(2+3)})}{f_2 f_3 - f_{(1+2+3)} (f_2 + f_3 - q_{(2+3)})}$$
[Equation #48]

$$q_{(2+3)} = \frac{f_1 f_2 f_3}{f_{(1+2+3)} (q_{(1+2)} - f_1)} + f_2 + f_3 - \frac{f_2 f_3}{q_{(1+2)} - f_1}$$
 [Equation #49]

$$q_{(1+2)} = \frac{f_3 (f_1 + f_2) + f_1 (f_2 - q_{(2+3)}) - f_1 (f_2 - q_{(2+3)})}{f_2 + f_3 - q_{(2+3)}}$$
 [Equation #50]

Example 1: Three lenses of a camera have focal lengths (from outermost): (1) 20 mm, (2) 30 mm, and (3) 40 mm. The first and second lenses are 10 mm apart and the second and third lenses are 30 mm apart. What is the combined focal length? Using equation #41:

$$f = \frac{20 \times 30 \times 40}{20(70) + 40(20) - 30(40)} = \frac{24000}{1000} = 24 \text{ mm}$$

Example 2: The lens system of example 1 is reversed. What is the new combined focal length for the "reversed" position? Using equation #46:

$$f = \frac{20 \times 30 \times 40}{40(50) + 20(0) - 10(40)} = \frac{24000}{1600} = 15 \text{ mm}$$

Example 3: The lenses of example 1 are used in the "normal" sequence. The two inner lenses are set 20 mm apart. Where should the outermost lens (lens #1) be placed to give a combined focal length of 16 mm? Using equation #45:

$$q_{(1+2)} = \frac{20 \times 30 \times 40}{16(-20)} + 20 + 30 - \frac{20 \times 30}{-20} =$$
$$= \frac{2400}{-32} + 20 + 30 + 30 = 80 -75 = 5 \text{ mm}$$

Example 4: With the same three lenses (as in example 1) arranged in the "normal" sequence, the two outer lenses are mounted as a unit 40 mm apart. How far should this unit be mounted in front of the rear lens to give a combined focal length of 240 mm? Using equation #44:

$$q_{(2+3)} = \frac{20(70) + 40(-10) - 24000/240}{20 + 30 - 40} = \frac{1400 - 400 - 100}{10} = \frac{900}{10} = 90 \text{ mm}$$

Example 5: With the same three lenses (as in example 1) in the "normal" sequence, the outermost lens is mounted 100 mm from the rear lens. Where should the middle lens be placed to give a combined focal length of 60 mm? Using equation #45, and letting

$$Q = q_{(2+3)} \text{ and}$$

$$q_{(1+2)} = 100 - Q,$$

$$q_{(1+2)} = 100 - Q = \frac{24000}{60(Q - 40)} + 20 + 30 - \frac{600}{Q - 40}$$

$$100 - Q = \frac{400 - 600}{Q - 40} + 50$$

$$Q^2 - 90Q + 1800 = 0$$

$$(Q - 60) (Q - 30) = 0$$

$$Q = 60, 30$$
hecking the combined foral lengths produced by

Checking the combined focal lengths produced by  $q_{(2+3)} = 60$  and 30, we find:

For 
$$q_{(2+3)} = 60$$
,  $f = \frac{24000}{20(70) + 40(-10) - 60(10)} =$   
 $f = \frac{24000}{1400 - 400 - 600} = \frac{24000}{400} = 60 \text{ mm}$   
For  $q_{(2+3)} = 30$ ,  $f = \frac{24000}{20(70) + 40(-40) - 30(-20)} =$ 

$$f = \frac{24000}{1400 - 1600 + 600} = \frac{24000}{400} = 60 \text{ mm}.$$

Hence, there are two positions to place the middle lens, each of which will give a combined focal length of 60 mm!

Example 6: The first two lenses (see example 1), having focal lengths of 20 and 30 mm, will not focus when separated by 60 mm:

$$f_{(1+2)} = \frac{20 \times 30}{20 + 30 - 60} = \frac{600}{-10} = -60 \text{ mm}.$$

Will these lenses as a system attain focus if they are placed 10 mm in front of a lens with focal length of 40 mm?

$$f_{(1+2+3)} = \frac{20 \times 30 \times 40}{20(70) + 40(-30) - 10(-10)} = \frac{24000}{1400 - 1200 + 100} = \frac{24000}{300} = 80 \text{ mm}.$$

- The three-lens system may be regarded as a twolens system in front of one lens, not as a twolens sytem behind one lens.
- Two lenses which together will not attain focus may, when combined with a third lens, form part of a three-lens focusing system.
- Reversal of a three-lens system rarely fails to change the focal length of the system.
- Any lens with a particular curvature on one side and a different curvature on the other behaves optically as two lenses with little or no spacing between their optical centers. The equivalent focal lengths of the two sides are difficult to determine without special instruments to measure the curvatures, and it is easier to find the "combined" focal length in each direction by direct experiment.
- If the lens system is extended over an appreciable interval, the addition of a concave lens will serve to shorten the combined focal length, and conversely, the addition of a convex lens will serve to lengthen the combined focal length.
- If the lens system is condensed into a short interval, the shortest focal length is obtained by addition of a lens of short focal length. Similarly, the focal length can be increased by the addition of a lens of very long focal length.

#### THE MICROSCOPE AS A LENS SYSTEM

Of all the instruments involving highgrade optical systems, the microscope and the camera are the two which have received the most attention in lens designs and the most refinement in engineering and manufacture. The economics of the optical microscope, which is used in high-school and university teaching and in research, has led to its perfection. A continuing market and competition between the leading manufacturers keep research and development thriving, and the optical systems of microscopes are in general superior to those in cameras.

The microscope can be adapted to provide the lens system for photomicrography. With the standardized design of current microscopes and their lenses, the adaptation for taking pictures of microscopic specimens is much easier now than at any time in the past. Many leading companies stock adapters and 35-mm backs to convert the microscope into a photomicrographic camera.

Brands of microscopes. -- Although the design of microscopes is essentially standardized, each company has its own size of camera body and its own sets of lenses. Parts are not regularly interchangeable between Zeiss, Wild, Bausch & Lomb, American Optical, or other makes of microscopes. Even though the differences in optics of objectives and eyepieces (oculars) and in the length of the tube on which they are mounted may not be readily seen, the tolerances in the combination are quite small and extremely critical -- apart from the fact that each company has its own dimensions and design of connectors and its own threading, purposely made to differ so that their objectives and eyepieces will not fit onto the body of a microscope made by a rival organization. Even within the products of one company, each set of objectives is made to be used only with a certain set of eyepieces.

In designing a photomicrographic camera using a microscope, therefore, it must always be borne in mind that each system of lenses is finely precisioned to work in optical harmony. The manufacturer's recommendations should be regarded, in most cases, as rules handed down by the world's finest optical specialists.

Optics of the microscope. -- The objective forms a real "aerial" image at a certain distance above the subject (specimen). When this aerial image coincides with the focal plane of the eyepiece, the observer "sees" a virtual image projected somewhere below the level of the subject. All modern microscopes have this eve-to-image distance fixed at 250 mm. Now if the eye does not intercept the rays coming out from the eyepiece, they can be projected 250 mm above the eye level position to form a real image. Hence, a camera with film placed 250 mm above the eye level position of the microscope will record the image of the specimen at the magnification produced in the virtual image. What was seen through the evepiece becomes what is recorded on the film.

For the subject to remain in focus when various objectives are used in the microscope, the distance from subject to aerial image must be kept constant. Of course, the tube to which lenses (both objective and eyepiece) are attached needs also to remain in the same position and its length is fixed. Hence, when the subject is in focus, the bottom of the tube (where the objective is attached) is a fixed distance above the specimen; this is the so-called "subject distance of the objective." The tube will extend a short distance above the level of the aerial image; this distance is called the "intermediate image distance of eyepiece." The subject-to-image distance is the subject distance of the objective plus the mechanical tube length minus the intermediate image distance of the eyepiece. The figures given below are for a Zeiss instrument.

Subject	dis-	Mechanical	Int	ermediate	e S	Subject-to-
tance of	ob-	tube	ima	ge dist,		image
jecti	ve	length	of (	eyepiece		distance
b	+	mtl	-	đ	=	a
45	+	160	-	10	Ξ	195 mm

For the image to remain in focus when eyepieces are exchanged, the eyepiece focal plane for each eyepiece must always coincide with the plane of the aerial image. That is, the eyepiece flange must also be located at a fixed distance (intermediate image distance of



the eyepiece) from the plane of the aerial image in the tube; the eyepiece mounts must be so designed that their focal plane is always at a fixed distance from their seating.

Hence, by careful designing of the mount for the objective, its aerial image will occur at a fixed position in the tube; and by designing of the mount for the eyepiece, its position of focal plane can be made to exactly coincide with the aerial image. In this way, objectives and eyepieces can be freely interchanged without re-focusing the microscope.

### 

NUMERICAL APERTURE

- Let  $\sigma'$  = apertural angle of objective lens (the angle formed when subject is in focus by intersection of optic axis with surface of subject (the center of the field in focus) and a line from this point to the edge of the aperture in the objective
  - NA = numerical aperture of objective
  - n = index of refraction of medium between subject and aperture of objective

 $NA = n \sin \sigma$  [Equation #51]

The greater the NA value, the more light admitted into the microscope and the brighter the image.

Increasing the NA value has the same general effect as opening the diaphragm of a camera lens (decreasing the *f*-stop number). Insofar as the microscope is being used as a camera lens, it gives less depth of field. For that reason, the ideal objective for photomicrography has a diaphragm.

NUMERICAL VALUES OF MAGNIFICATION AND FOCAL LENGTHS

- Let  $m_m$  = numerical value of magnification of microscope
  - m<sub>o</sub> = numerical value of magnification of objective (scale of image formed by objective)
  - m<sub>e</sub> = , americal value of magnification of eyepiece (scale of image formed by eyepiece)

$$f_{\rm m}$$
 = focal length of microscope lens system

 $f_{o}^{m}$  = focal length of objective

- $f_{\rm e}$  = focal length of eyepiece
- ot1 = optical tube length (not the same as mechanical tube length!)
- mtl = mechanical tube length; fixed by the manufacturer to a specific length; the distance from the attaching mount of objective to the seating of the eyepiece.
  - **a** = distance from subject to aerial image
  - d = distance from aerial image to seating
     of eyepiece
  - b = distance from subject to attachment
     of objective
  - q = distance between optically equivalent center points of objective and eye-piece.

By the construction of the microscope, when it is in focus:

#63]

$$mtl + b - d = a$$
 [Equation #52]

For each make of microscope, each of these factors has a *fixed* value.

For the total system:

 $m_{\rm m} = 250/f_{\rm m}$  [Equation #53]

 $f_{\rm m} = 250/m_{\rm m}$  [Equation #54]

$$f_{\rm m}m_{\rm m} = 250 \text{ mm}$$
 [Equation #55]

The product of focal length x numerical value of magnification in any standard microscope is 250 mm. Thus, when in focus, the virtual image seen in normal microscope use is 250 mm below eye level; and when the image is projected beyond (above) the eyepiece, it forms a real image (suitable for registration on film) at 250 mm above eye level.

For the objective:

m <sub>o</sub>	=	$otl/f_o$	[Equation	#56]
$f_{ m o}$	=	otl/m <sub>o</sub>	[Equation	#57]
$f_0 m_0$	=	otl	[Equation	#58]

Example 1: A 20x objective with a focal length of 8.3mm produces an optical tube length of:

 $20 \times 8.3 = 163 \, \text{mm}$ 

The optical tube length is the product of the focal length of the objective x its numerical value of magnification.

For the eyepiece:

$m_e =$	250/f <sub>e</sub>	[Equation	#59]
$f_{\rm e}$ =	250/m <sub>e</sub>	[Equation	#60]
$f_{\rm e} m_{\rm e} =$	250 mm	[Equation	#61]

Example 2: A 10x eyepiece has a focal length of:

 $250/10 = 25 \, \text{mm}$ 

The product of focal length of the eyepiece x the numerical value of its magnification is constant, fixed in all standard microscopes at 250 mm.

For the system;

 $m_{\rm m} = m_{\rm o} \times m_{\rm e}$  [Equation #62]

Example 3: A 20x objective used with a 7.5x eyepiece produces a magnification of

 $20 \times 7,5 = 150x$ 

The numerical value of magnification of a microscope is the product of the numerical values of magnification of its objective and eyepiece.

Combining equations #62, #56, #59, and #53:

Example 4: A 45x objective having focal length of 4 mm is used with a 10x eyepiece. What is the focal length of the combination?

From equation  $#58: otl = 45 \times 4 = 180 \text{ mm}$ 

From equation #60:  $f_e = 250/10 = 25 \text{ mm}$ From equation #63:  $f_m = \frac{4 \times 25}{180} = \frac{5}{9} \text{ mm}$ 

The same result could be obtained from the numerical values of magnification (using equation #62):

$$m_{\rm m} = 45 \times 10 = 450 \times f_{\rm m} = \frac{250}{450} = \frac{5}{9} \,{\rm mm}$$

- The focal length of the microscope system is the product of the focal length of the objective x focal length of the eyepiece divided by the optical tube length.
- In the optical system of the microscope, q, the separation of objective and eyepiece equivalent centers, is always negative. Thus:

 $-q = ot1 - f_0 - f_e$  (see sketch) [Equation #64] From this we can derive equation #63 from #38:

$$f_{\rm m} = \frac{f_{\rm o} f_{\rm e}}{f_{\rm o} + f_{\rm e} - q}$$
  
-q = otl - f\_{\rm o} - f\_{\rm e}  
$$f_{\rm m} = \frac{f_{\rm o} f_{\rm e}}{f_{\rm o} + f_{\rm e} + otl - f_{\rm o} - f_{\rm e}} = \frac{f_{\rm o} f_{\rm e}}{otl}$$
  
Example 5:  $f_{\rm o} = 16 \,\mathrm{mm} \, m_{\rm o} = 10 \,\mathrm{x}$   
 $f_{\rm e} = 25 \,\mathrm{mm} \, m_{\rm e} = 10 \,\mathrm{x}$   
otl = 16 x 10 = 160 \,\mathrm{mm}

From equation #64:

$$-q = 160 - 16 - 25 = 119 \,\mathrm{mm}$$
$$f_{\rm m} = \frac{16 \times 25}{16 + 25 + 119} = \frac{16 \times 25}{160} = 2,5 \,\mathrm{mm}$$

The same answer is obtained from equation #63:

$$f_{\rm m} = \frac{16 \times 25}{160} = 2.5 \,\rm mm$$

Or the same answer can be got from equation #62:

$$m_{\rm m} = 10 \times 10 = 100 \times f_{\rm m} = 250/100 = 2.5 \, {\rm mm}$$

=

							Eyepi	eces			
Objec	tives				Huygenian Hyper- plane					-	
	f		ot1	$f_{ m e}$	50	33.3	25	20	16.67	12.5	$f_{e}$
Туре	_(mm)		(mm)	_me	_ <u>5x</u> _	7.5x	<u>10 x</u>	12.5x	15x	20x	e
	48	2x	96		10	15	20	25	30	==	
	32	4x	128		20	30	40	50	60	==	
ic	16	10x	160		50	75	100	125	150	200	
mat	8	21x	168		105	157	210	263	315	420	
hro	4	43x	172		215	320	430	537	645	860	
Ac	3	60x	180		300	450	600	750	900	1200	
	1.9	97x	184		485	727	970	1212	1455	1940	
	1.8	100x	180		500	750	1000	1250	1500	2000	
					Compensating						
	f		otl	$f_{e}$	50	33.3	25	20	16.67	12.5	10
Туре	(mm)	m <sub>o</sub>	(mm)	me	5x	7.5x	_10x	12.5x	<u>15x</u>	20x	25x
	16	10x	160		50	75	100	125	150	200	250
ŗ	8.3	20x	163		100	150	200	250	300	400	500
mat	4	<b>4</b> 5x	180		225	338	450	562	675	900	1125
hro	3	61x	183		305	457	610	762	915	1220	1525
poc	3	62x	186		310	465	620	775	930	1240	1550
A	2	90x	180		450	675	900	1125	1350	1800	2250
	1.5	120x	180		600	900	1200	1500	1800	2400	3000

Table of Microscope Magnifications

Numerical Aperture. -- The equation for NA is given above (equation #51). In designing and making objectives, NA is of great concern. Each manufacturer strives for the maximum NA possible without introducing serious aberrations in the marginal region of the lens.

Theoretically, the ability of the objective to resolve detail depends upon its NA and the wavelength of the light used:

lines resolved =  $2 \times NA / wavelength$  of light

This is the theoretical or potential resolving power of the objective; how closely it is actually approached depends upon the skill and precision in the lens manufacture. As shown in the table below, resolving power is greatest for the short wavelengths (blue and violet) and

for the objective with the greatest NA.

Theoretical	Resolving	Power	of	Objectives	
-------------	-----------	-------	----	------------	--

Wavelength in microns						
NA	Red .700	Orange .615	Yellow .590	Green .545	Blue .485	Violet .430
.25	715	815	845	915	1020	1165
.50	1430	1625	1695	1830	2040	2325
.75	2145	2440	2540	2750	3060	3490
1.00	2860	3255	3390	3665	4080	4650
1.25	3570	4065	4235	4580	5100	5810
1.50	4285	4880	5085	5500	6120	6975

\* Lines per mm

Resolving power is not the only factor related to NA. Inasmuch as the numerical aperture is a function of the actual apertural opening and the objective-to-subject distance, it acts in part like an actual diaphragm in a camera lens. Thus the brightness of the image is directly proportional to the square of the NA.

Similarly, just as increasing the diameter of the opening in a camera lens will decrease the depth of field, so increasing the NA value will also decrease the depth of field. It also decreases the flatness of the field by introducing aberrations in the increased size of the effective portion of the lenses in the objective.

Effects Produced by Increased NA

Factor	Effect
Resolving power	Proportional increase
Brightness of image	Proportional to square of NA
Depth of field	Decrease
Flatness of field	Decrease

It soon becomes apparent that using the microscope objective as part of a camera lens system counteracts the goals of the optical physicists who design microscopes. For normal microscopy, the best lens has the highest NA (other factors being equal), with highest resolving power and highest brightness of image produced. To achieve this, depth of field and flatness of field have been sacrificed. This is one of the ways in which photomicrography goes against the basic principles and premises of good microscopy.

Kinds of lenses. -- Uncorrected lenses have many of the inherent defects or aberrations listed below, which make them unsuitable for good microscopy and especially unsuitable for photomicrography.

Basically, there are two types of corrected lenses: achromatic and apochromatic. But there are also varieties according to the degree of correction and just what has been corrected.

(1) ACHROMATIC -- focal distances of two colors,

such as blue and red, are made equal. Other colors fall near these two near-end members of the spectrum. These lenses are suitable only for transmitted light; they are not for photomicrography. They are chromatically corrected for two colors and spherically for one color. Such objectives are designed for visual work, and are best with yellow-green -- the particular color most effective with the eye. Because these lenses are not fully corrected, they are not intended to be used with the usual eyepieces of higher than 12.5x magnification. Achromatic objectives are used with low-power (5x to 12.5x) eyepieces of Huyghenian design or with higher-power (20x) eyepieces of Hyperplane design.

The combination of two kinds of glass in the achromatic lenses alleviates some of the problems of color differences in the image, but it does not eliminate them. Even with the best correction, the achromatic objective retains problems with residual colors of the secondary spectrum.

(2) APOCHROMATIC -- refined lenses of highest order corrected to eliminate abberations chromatically for three or more colors and spherically for two colors. The colors of the secondary spectrum are eliminated completely in a good apochromatic lens, and only faint suggestions of tertiary spectrum remain as residual color. Such lenses are best suited for photography. They are used with compensating eyepieces to correct differences in magnification produced by the objective.

(3) SEMI-APOCHROMATIC -- improved over plain achromatic lenses, but not as fully corrected as apochromatic.

(4) PLANACHROMATIC -- improved chromatic correction, and elimination of curvature of field.

(5) PLANAPOCHROMATIC -- focal distances made equal for several colors. Some are made with very high NA.

(6) MICROTESSAR or LUMINAR -- lenses specially made for photomicrography without an eyepiece. The shorter focal lengths (16-, 25-, and even 40-mm) in these lenses may be combined with specially designed eyepieces which optically complement their corrections. Lens aberrations. -- No lens is perfect. The aberrations mentioned here apply to regular photographic lenses as well as to microscope lenses used in photomicrography. In the microscope, the chief characteristics of the image are controlled by the objective and the eyepiece serves mainly to enlarge the image so produced and project it onto the film. Hence, the "defect" of the objective is of prime importance.

(1) SPHERICAL ABERRATION -- longitudinal variation of image position for different zones of the lens. Rays from a subject on the optic axis enter the lens at numerous points and are refracted to cross the optic axis (on the image side) at different points, usually at successive points as the rays pass through more distant parts of the lens. In uncorrected lenses, the rays which pass through the outer parts of the lens fall short of the focal point of rays passing through the lens near its center. In corrected lenses, rays passing through the margin of the lens are made to refract at the focal point of the rays passing near the center, but rays through part of the intermediate zone may fall short of the focal point; this is called zonal aberration. The lens can be tested for forms of spherical aberration by examining the image of a bright point source focused at the center of the field. With the lens closed down, the image is brought into sharp bright dot (tiny circle); then, with the lens open and part (including the center) shielded, see if the dot still has good definition.

(2) COMA -- variation of image size for different zones of the lens. Coma practically ceases to exist near the axis of even an average lens, but it increases steadily for images toward the edge of the field. The rays from a bright point, entering the lens at an angle from the optic axis, are refracted in such a way that those through the outer parts of the lens form a circular image, but those through the rest of the lens are refracted less and fall beyond the circle (farther from the center of the field) to produce a comatic (cometlike) form of image.

(3) LONGITUDINAL CHROMATIC VARIATION -- longitudinal variation of image position for different colors. In an uncorrected lens, the focal point varies for different colors: violet is the shortest and red the longest, with blue, green, yellow, and orange between. By using two kinds of glass, as in an achromatic lens, an attempt is made to bring two colors to unite at a common focus. When this is done, the intermediate colors fall closer to the lens than the common focus and the extreme colors fall beyond. In apochromatic lenses, three colors are brought to a common focus, or as nearly as possible to it. All determinations are made for a point source of light at a fixed distance out from the lens on the optic axis.

(4) CHROMATIC DIFFERENCE OF SPHERICAL ABERR-ATION -- different patterns of longitudinal variation of image position for different zones of the lens with different colors. The spherical aberration (see item 1 above) may not be the same for one color as for another. With the combination of different glass compositions used to produce achromatic or apochromatic lenses, one color may have a slight zonal aberration whereas another color may have very strong spherical aberration. The perfect lens would have all parts of the lens reach a common focus for all colors, but such a lens has never been produced. The best lenses reduce the degree of spherical aberration for two or three selected colors.

(5) CHROMATIC DIFFERENCE OF MAGNIFICATION -variation of image size for different colors. This is not the same as longitudinal chromatic aberration, in which the position of the focal point varies in different colors at the center of the field. This concerns the different image sizes produced by different colors, and manifests itself by colored fringes around the edges of the image -- becoming stronger near the corners of the field. These fringes register on black-and-white film as blurred outlines. The defect is not serious in modern microscope objectives, but it is not decreased in any way by stopping down the lens.

(6) DISTORTION -- variation of magnification in different parts of the field. If distortion is present, the magnification is not constant over the field; the outer parts may be magnified more or less than the central part. If the periphery is magnified less than the center (as is obvious in a picture of a divided scale extending across the field), the image of a square will have convex sides and appear "barrel-shaped." On the other hand, if the periphery is magnified more than the center, the image of the square will have concave sides and assume the so-called "pincushion" shape. Distortion is not affected by stopping down the lens. Lenses with distortion reduced to a minimum are called <u>rectilinear</u> or orthoscopic lenses.

(7) ASTIGMATISM -- a longitudinal separation between the images of radial and tangential lines in the field. This defect does not exist on the axis of a well-centered lens, but may increase rapidly in oblique rays and be manifest near the edges of the field. If a wheel is photographed with an astigmatic lens, the radial spokes may be in focus while the tangential rim is blurred, or vice versa.

(8) CURVATURE OF FIELD -- curvature of the 'field surfaces" obtained by joining up the radial and transverse astigmatic images over the entire field. If all the tangential focal lines (circles) are joined from a plane subject (perpendicular to the optic axis, of course), they will reach their best definition on a curved surface called the "tangential field curve" of the particular lens. Similarly, the radial focal lines all lie on a "radial field curve" or the so-called "sagittal field curve" of the lens. These two fields coincide at the center of the field, since all astigmatism vanishes there. Stopping down the lens does not affect the positions of these curved planes, but because it increases the depth of field and shortens the focal lines themselves, for practical purposes it reduces the effect of astigmatism on the image.

N	ear optic axis	Near edge of field
1	Spherical aberra- tion	l Coma 2 Astigmatism
2	Coma	3 Curvature of field
3	Longitudinal chrom- atic aberration	4 Distortion
4	Chromatic difference of spherical aber- ration	5 Chromatic difference of magnification

Common Lens Aberrations

<u>Combined microscope lens system.</u> --If the microscope is to be used normally (to magnify specimens for visual examination), better results are obtained in modern instruments by a high-power objective and a low-power eyepiece than by a low-power objective and a high-power eyepiece. This is true because the high-power objectives have relatively higher NA values and consequently brighter images and higher resolution. For photography, however, the reverse is true. Better results are obtained with a low-power objective and a highpower eyepiece, because such a combination yields greater depth of field -- and the reduced brightness of image is no appreciable handicap in exposure of the film.

Most makes of microscopes, unfortunately, have few suitable objectives. The manufacturers have devoted little effort to development of widefield low-power objectives with diaphragms. Perhaps this situation prevails because many photographic uses of the microscope involve thin sections (slides) illuminated by light transmitted from below and presenting a minimum of depth (thickness).

One combination which is manufactured by Leitz includes the Pl Plano (achromatic) 1x objective (NA = 0.04; focal length = 33 mm; free working distance = 30 mm) provided with a diaphragm and the GF Periplan Widefield eyepieces available in 10x, 12.5x, 16x, and 25x. With these basic lenses, the Leitz microscope can be equipped with micro camera attachment giving 1/3x or 1/2x with a focusing telescope to show the precise focus and coverage on the film in a Leica camera body. Thus, with Pl 1x objective, GF 10x eyepiece, and 1/3x attachment, the image on film will be  $1 \times 10 \times 10^{-1}$ 1/3 = 3.33x; or with the same objective, GF 25x eyepiece, and 1/2x adapter, the image on film will be  $1 \ge 25 \ge 1/2 = 12.5 \ge$ . Intermediate combinations are also possible (without re-focusing the microscope):

Magnifications on Film using Pl lx Objective

Eyepiece	1/3x Adapter	1/2x Adapter
GF 10x	3.33x	5.00x
GF 12.5x	4.17x	6.25x
GF 16x	5.33x	8.00x
GF 25x	8.33x	12.50x

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