by

ROY M. ALLEN

Photomicrography

SECOND EDITION

PHOTOMICROGRAPHY

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SECOND EDITION

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Preface to the Second Edition

Advances in microscopy along many lines have been numerous since the publication of the first edition. New designs of microscopes, cameras, and accessories have made much earlier apparatus obsolete, even though basically the older equipment is in most cases just as efficient when properly used. The trend in photographic equipment has been largely toward universality of application, combined with automatic operation.

Among the advances in theoretical and applied microscopy can be mentioned phase and interference microscopy, electron microscopy, the widespread use of color photography, and the application of polarized light to many types of investigation not previously considered practical or useful. The introduction of Polaroid and its frequent substitution for the more expensive types of calcite prisms has helped to make the use of polarized light more general.

Much of the new apparatus has been so designed that a microscopist no longer need be trained in optical theory when it comes to the operation of his microscope or camera. Integral light sources, properly aligned for critical illumination, are now available in many forms for those who desire to use them. Flexibility in accessory apparatus, at least in the more elaborate equipments, enables one to do all types of microscopical work—light field, dark field, polarized-light, phase microscopy, and photography in color in all sizes of film from 16- and 35-mm. up to the capacity of the camera provided—all this with a complete range of magnifications—as well as copying, enlarging, reducing, and other ordinary types of photographic work occasionally required in photomicrographic work.

In the optical field there is now a definite trend toward the application of the method suggested by Sir Isaac Newton for eliminating chromatic and spherical aberrations through the use of spherical mirrors as objectives. It can be predicted that future developments along this line will be made and wider application of this type of objectives will result.

Another development in the sister art to photomicrography, microphotography, has come about as a result of the extensive employment of V mail during World War II. The advantage of reduced copies of publications, books, records, etc., to meet the evergrowing demands upon storage space has brought the art of microphotography into commercial prominence. This has resulted in the development of equipment for producing the reduced copies and means for facilitating the reading of them.

This new edition of Photomicrography has taken cognizance of these modern trends and illustrates some (it is not possible to include all) of the changes in design of microscopes and photomicrographic equipment. New chapters have been added to cover phase and interference microscopy as well as electron microscopy, which was in its infancy at the time of publication of the first edition. Other portions of the text have been added to and brought up to date when necessary. It has been the aim throughout to explain developments, where optical principles are involved, in terms comprehensible to individuals without technical training who may be called upon to use the latest equipment. Portions of the earlier edition which are still pertinent have been left unchanged. Illustrations of older equipment, now obsolete but still in general use, have also in several instances been allowed to remain.

It should be pointed out that no attempt has been made to cover all the equipment manufactured or supplied by the many companies in this field. Much that has not been mentioned is of the highest quality. The deciding factor in determining what should be shown has been the publicity given in the past to manufacturers whose products have been in general use. Well-known British microscopes and equipment are unsurpassed in both mechanical and optical performances, and such names on a microscope as Watson, Baker, Beck (to mention a few) are guarantees of quality equal to the best; but these instruments are seldom to be found in use among microscopists in the United States. Those who do possess English instruments or optics (for the most part amateurs) are invariably enthusiastic in their praise of them. Yet for some reason the British manufacturers in general have made little or no attempt to invade the American market; hence these are given but brief mention.

Thanks are due to all the manufacturers and suppliers of microscopical and associated equipment (including the American agents for foreign companies) for their wholehearted cooperation with the author in furnishing catalogues, information, suggestions, and photographs. These include Bausch & Lomb Optical Company, American Optical Company, Eastman Kodak Company, E. Leitz, Inc., Carl Zeiss (Zeiss-Winkel), Ercona Corporation (East German Zeiss), Wm. J. Hacker & Company (agents for Reichert), the R. Y. Ferner Company (U.S. representative for Cooke, Troughton & Simms), Silge & Kuhne, Photovolt Corporation, Fish-Schurman Corporation, Fisher Scientific Company, Radio Corporation of America, Philips Electronics, Inc., Farrand Optical Company, and many individuals in the various companies who have given personal help.

September 1958 ROY M. ALLEN

Preface to the First Edition

Rapid modern advances in the application of the microscope to problems of all sorts, both research and commercial, have naturally resulted in a simultaneous development of the art of photomicrography. The latter can no longer be classed as a hobby, indulged in only by a small group of amateur microscopists; it has become an essential adjunct to the use of the instrument itself in practically every scientific and commercial field.

In spite of this, authoritative reference books on the subject of photomicrography are lacking. Whether this is the cause or the effect of a common idea that expert knowledge is unnecessary for taking pictures through the microscope is immaterial. The fact remains that photomicrographs (of a sort) are being taken daily by hundreds, if not thousands, of microscopists, in every line of microscopic endeavor, whose sole qualification for the task is a fundamental knowledge that the image formed by a microscope can be projected onto a sensitive photographic film and be reproduced thereby.

That better pictures can be taken as one's experience and knowledge of the technique increase is to be assumed, as is also the fact that the majority of individuals can learn more rapidly and easily by assimilation of the published experiences of others than by the slower process of personal plodding. This is the reason for the existence of this book.

Although the close relationship between visual work with the microscope and photomicrography would seem to have warranted inclusion of the latter in the author's book, *The Microscope*, this was not feasible because of the large amount of material which would have had to be included to cover the subject in a thorough manner. The alternative was the complete segregation of the two subjects and their publication separately.

A work dealing primarily with the photographic aspects of microscopy cannot, of course, undertake an exhaustive discussion of the elementary optics of the microscope to the same degree as a book primarily on the instrument itself. Some knowledge on the part of the microscopist must be assumed. On the other hand, it is desirable that a work on photomicrography be sufficiently self-contained to be understood without constant reference to outside matter. This implies that some duplication of subject matter common to both visual and photographic work must occur – so much, at least, as may have a bearing on the production of ideal photomicrographs.

The material contained in the present volume is derived almost exclusively from the author's personal experience, which has extended into practically every known application of the microscope, and is passed on in the hope that it will prove for many a substantial short cut to a working knowledge of photomicrography.

For the benefit of those already familiar with the use of the microscope, the text has been so arranged that only matters of direct interest need be considered, without wading through irrelevant matter. On the other hand, beginners can follow through each chapter in the sequence presented, and after becoming acquainted with the subject from the standpoint of basic principles and the various ways these have been worked out in mechanical design, can take up the practical technique of photomicrography. The chapter on photographic processes has been added to provide information on this phase of the work for those who may not have had previous experience with them.

I wish to express my appreciation of the wholehearted cooperation extended to me by various companies in providing cuts of the various apparatus and equipment shown. These include, The Bausch & Lomb Optical Co., Spencer Lens Co., E. Leitz, Inc., Carl Zeiss, Inc., Eastman Kodak Co., Radio Corporation of America, Cooke, Troughton & Simms of York, England, and others.

R. M. A.

June 1941

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Fundamental Principles of Photomicrography

Definitions

The word *photomicrograph* is a compound of *photograph*, a picture produced through the instrumentality of light, and *micrograph*, an enlarged reproduction of a minute object. Originally "micrograph" meant only a drawing, made by free hand, or by means of a pantograph, as these were the only methods known for producing the enlarged image. The application of photographic processes of reproduction to the microscope provided a new method of securing enlarged pictures of minute objects, hence the combined word *photomicrograph*. This compound word necessarily is limited to mean a picture taken through a microscope, by means of light acting on a sensitive emulsion, but the word *micrograph* used alone has had to be broadened in meaning to include pictures of minute objects *either* drawn by hand or produced through photographic processes.

As a matter of fact, photomicrography is so rapidly replacing hand drawings (made by means of a camera lucida attached to a microscope) that it is probably only a question of time until the term *micrograph* will come to mean *only* a photomicrograph.

Another term introduced to cover a particular class of photomicrographs is *photomacrograph* (incorporating the Greek word for "long," *makros*), meaning a photographic image of a relatively large object magnified only a few times, i.e., not over ten diameters. A "macrograph" is, then, a *slightly* enlarged picture of an object.

Sometimes the word *microphotograph* is used for photographs taken with the microscope, but this word is used incorrectly, for it means "a minute photograph," which must be examined as a microscopic object, under the microscope, in order to observe the details of the picture. The relation of microphotographs to the microscope, and the method of producing them, will be considered in Chapter 8.

Relation of Photomicrography to Ordinary Photography

The possibility of taking pictures by means of the microscope is dependent upon the fact that basically there is no difference between a camera lens and a microscope. They both obey the same laws of optics in producing an image of a properly illuminated object. As a matter of fact, photomicrography and ordinary photography merge so perfectly into each other that it is difficult to say just where one leaves off and the other begins. The best place, therefore, to commence a discussion of the optics of photomicrography is with the camera lens.

In every case, a camera lens must be of what is known as the "positive" lens type, i.e., one that is thicker in the center than at the periphery. Only a positive lens (the term of course includes compound lenses functioning together as a single positive lens) can produce a *real* image such as is required for photographic purposes. Negative lenses are those which are thinner through the center than at the periphery. These, or combinations of lenses which have a resultant effect similar to a negative lens, cannot produce a photographic image. The image in this case is known as a *virtual* image.

When an object is located a great distance from a camera lens, ordinarily known as "infinity," the rays from the object are brought together by the lens to form an image of the object in what is called the focal plane of the lens; the distance of this plane from the optical center of the lens is designated the *focus* * of the lens. For all practical purposes, the distance "infinity" can be considered as anything over 200 times the focal length.

The size of the image bears a definite relation to that of the object, which is the ratio of the distances between the lens and the object, and the lens and the image. Thus the image of an object located 200 times the lens focus from a camera will be 1/200 actual size. Only when the object is located at infinity will the image plane be distant from the lens the exact focal length of the latter. As the object comes nearer, the image plane moves farther away from the lens, and the ratio between the object size and image size changes accordingly. This, of course, is why cameras must be focussed for the proper object distance, if one is to obtain sharp pictures.

* Technically, this is known as the *principal focus*. Actually, every lens has two foci, one on each side of the lens. These are called *conjugate foci*. The conjugate focal point corresponding to the principal focus is located at infinity. As it comes nearer the lens, the corresponding conjugate focus recedes.

When the object is brought to a certain distance from the lens it is found that the image plane has receded to a point where it is the same distance from the lens as the object distance. This distance is found to be exactly twice the focal length. Under this condition, the image size is identical with the object size. With ordinary hand cameras this cannot be accomplished, because not enough adjustment or bellows length is provided; therefore, if photographing an object full size is desired, a special long-bellows camera is necessary.

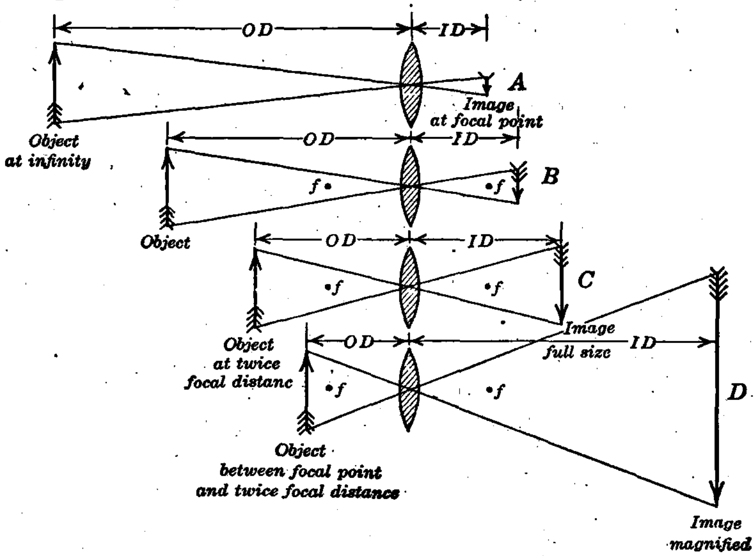

FIG. 1. Relationship between Image size and Object size. (I : O :: ID : OD)

If the bellows is sufficiently long, it is not obligatory to stop when full size has been reached; bringing the object still nearer results in moving the image plane farther away and the image then becomes *greater* than the object, or *magnified*. Thus the same camera lens becomes in effect a photomicrographic lens producing possibly a magnification of several diameters. These various conditions are shown diagrammatically in Figure 1.

Thus far we have not taken into account the effect of a change in the focal length of the lens on image formation. Here the parallel between a camera lens and a microscope becomes still closer: Let us study a few examples and apply the simple mathematical ratio to the object and image size.

Assume an object 10 inches high located 100 feet distant to be photographed by a camera equipped with a 6-inch (½ ft.) lens. Its photographic image is 10″ × 1/200 = 1/20 of an inch high. Using a 12-inch (1 ft.) lens under the same conditions, the image size would be 10″ × 1/100 = 1/10 inch. In other words, when the distance is fixed, the size of the image can be increased substantially in proportion to the increase in the focal length of the lens employed.

This will hold more or less true as the distance of the object from

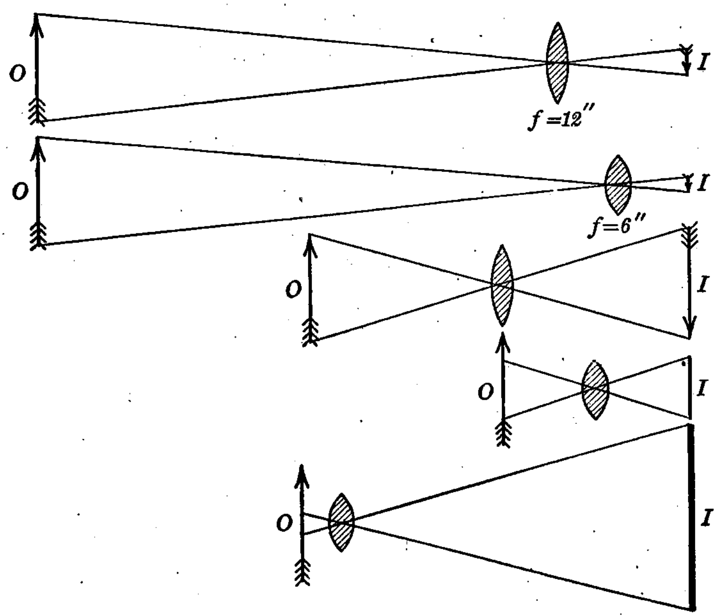

FIG. 2. Effect of the focal length of the lens on image size

the camera is decreased. But with extreme nearness of the object other factors enter the problem. We have already seen that any lens is capable of producing a full-sized image when the object is distant twice the focal length of the lens from the latter. With a 12-inch lens this occurs when the object is 24 inches away. It also requires a camera bellows length of 24 inches, while with a 6-inch lens the object yields a full-sized image with an object distance of 1 foot and only a 12-inch bellows length is required. Should we wish to make a several times enlargement of the object we would discover that a 12-inch lens is almost prohibitive because of the excessive bellows length required for the camera; the 6-inch lens is far superior in this respect Assum ing that our maximum bellows length is limited to 36 inches, it is evident that employing a still shorter-focus lens, say a 3-inch, would enable a much higher magnification to be obtained. Going to a ½-inch lens would provide a possible magnification around 70 diameters. This has no bearing at all on the fundamental law; it only means that should we wish to secure a similar magnification with a 12-inch lens, we would require a bellows in the form of a tunnel about 75 feet long.

Conditions arise both in ordinary photography and photomicrography where lenses of longer focus are required to meet certain conditions. These conditions, as will be pointed out later, have to do with the relative area of the object which must be included in the photograph; the longer-focus lens accommodates a correspondingly greater area. Figure 2 illustrates graphically the effects of variation in the focal length of the lens on the image size and the way in which the area of the object included in the field of view is restricted by the use of shorter focus lenses.

A question might naturally arise in connection with the statement already made that a ½-inch lens used with a bellows extension of 3 feet will yield a magnification of some 70 diameters: "Why not use a still longer bellows and obtain magnifications of 100 or even several hundred diameters, with the same lens?" The answer to this question provides the justification for introducing the microscope and microscope lenses into the picture. It might appear sufficient to dismiss the suggestion of magnification by means of a long bellows wholly on the basis of its mechanical impracticability, but this is not the primary objection to it.

In the first place, the ½-inch lens proposed is already out of the class of ordinary camera lenses and can properly be considered a microscope lens. There is, in this respect, a considerable overlapping in the region of short-focus camera lenses (even excluding motion-picture cameras) and long-focus photomicrographic lenses. The former extend down to less than 2 inches and the latter up to at least 4 inches (100 mm.). There is, however, as will be demonstrated later, usually a radical difference in the design of the lenses for the two purposes.

In the second place, a more obvious solution would be to use the same bellows and reduce the focal length of the lens. A ¼-inch lens used on a 36-inch bellows would double the magnification obtainable with a ½-inch lens. This is the basis of the actual procedure employed in the production of highly magnified photomicrographs. Merely shortening the focal length of the lens, however, would not be satisfactory; there are certain optical laws inherent in the problem of producing magnified images of an object in which structural details are to be revealed in direct proportion to the amount of enlargement secured. An understanding of these laws and the manner in which they are met in the design of microscope lenses is essential to a thorough knowledge of photomicrographic technique. But before they can be taken up a discussion of some fundamental optics is in order.

Spherical and Chromatic Aberrations in Lenses

Unfortunately a positive lens constructed from a single piece of glass is not satisfactory for either high-quality photographic or photo-

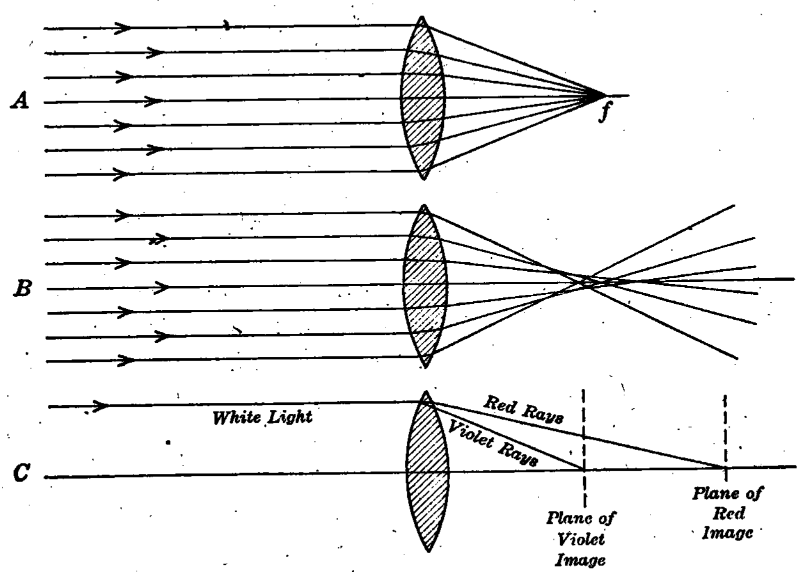

FIG. 3. Spherical and chromatic aberrations

micrographic work. This is because certain aberrations are present which interfere with the production of a perfect image.

The ideal condition is represented in Figure 3*A*, where all the rays proceeding from a point source of light located at infinity are brought together to form a point image. But what actually happens is that the rays passing through the outer zones of the lens are refracted excessively so that they come to a focus nearer the lens than those through the central zone. This effect, shown in Figure 3*B*, results