The Technique of PHOTOMICROGRAPHY

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FOREWORD

by DR. JOHN FARQUHARSON, F.R.I.C.

Managing Director, Beecham Research Laboratories Ltd.

FOR very many years the microscope has been one of the most potent tools in the hands of the scientist. Photomicrography, which may be regarded as a means of obtaining a permanent record of the field viewed in the microscope, has of recent years become a most important adjunct to the work of the research laboratory. This has led to the development of most elaborate photomicrographic apparatus. It is possible, however, to secure first-class pictures with a simple arrangement of lamp, microscope and camera, provided that due attention is paid to the detail of the light-supply. This matter of illumination is the main reason for the failure of most amateur and many professional photographers to obtain good results.

Douglas Lawson has made himself a master in the field of photomicrography, and this book is designed to impart his experience to others. Due attention is paid to the theoretical aspects of the subject, and the practical application is well documented. The result is a balanced and comprehensive book which will be most valuable to amateur and professional alike.

The publication of this book should introduce photomicrography to a much wider public, and should enable readers to use microscope and camera with confidence and success.

J. F.

PREFACE

I HAVE written this book with a view to providing some help and inspiration for laboratory workers and amateur and professional photographers who wish to embark upon photomicrography. The photomicrographs illustrated, which range from low- to high-power magnifications and include a variety of subjects, have been obtained by use of numerous techniques, with the exception of electron photomicrography. I have also referred to infra-red, ultra-violet, X-ray, fluorescence and interference photomicrography, which are used in industry and research and which play an important part in medical photography.

It is hoped that *The Technique of Photomicrography*, written after many years of work with the microscope in the laboratory, will be of assistance to those already experienced in research, medicine, industry, natural history, etc., and to all scientific branches of Her Majesty's Forces.

No pen, pencil or brush, however skilfully used, can produce the accuracy of a photomicrograph. In order to master the technique of photomicrography the worker must have a knowledge of the optical layout of the microscope and of all methods used to produce a photograph through its lenses.

In my research work I have examined rare and otherwise unobtainable specimens with my microscope and camera, and I wish to thank Dr. J. Farquharson, F.R.I.C., for his permission to reproduce illustrations of some of these uncommon subjects.

I collected and mounted much of the illustrated material while I was engaged in compiling a library of several hundred fixed slides. My keen interest in natural history has contributed to enlarging the collection.

I would particularly like to thank Mr. R. G. W. Ollerenshaw, M.A., M.R.C.S., F.I.B.P., F.R.P.S., for reading the manuscript and checking the drawings, and for his helpful suggestions. To Professor S. Tolansky, D.Sc., F.R.S., and Dr. M. H. F. Wilkins I should like to express my gratitude for their supplying me with photomicrographs of less common subjects.

I am indebted to R. & J. Beck Ltd., Mr. E. J. G. Beeson (A.E.I. Lamp & Lighting Co. Ltd.), Boone Instrument Co., New York, General Electric X-ray Corporation, Milwaukee, Langham Photographic Instruments Ltd., E. Leitz (Instruments) Ltd., Philips Electrical Ltd., Photovolt Corporation, New York, C. Reichert A.G., Vienna, Sylvania Electric Products, Inc., New York, W. Watson & Sons Ltd. and Carl Zeiss, Jena, for the use of illustrations and information; and to the Editor of *Endeavour* (Imperial Chemical Industries Ltd.) and C. Reichert A.G., Vienna, for the use of colour blocks.

I should like to thank my sister Dulcie for her readiness to help at all times, Mr. K. Austin for making several pieces of equipment for me, and Mr. E. Broome for checking the bibliography.

Finally, I wish to express my gratitude to the publishers for their co-operation, which has been enjoyed at all times.

D. F. L.

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INTRODUCTION

At the beginning of the fifteenth century the simple and compound microscopes then in use were known as fleaglasses and flyglasses, probably because fleas and flies were the first subjects to be viewed through them. As photography had not then been invented, sketches were the only means of recording the wonders of nature discovered through the microscope. The first compound microscope was simply an inversion of the telescope and was made by Galileo in 1609; little interest, however, appears to have been aroused. About twenty years later Kepler produced a better form of microscope, which was capable of clear low-power magnifications. It is on record that the capabilities of this instrument were most impressive.

In the middle of the fifteenth century a prominent worker by the name of Cesi used his microscope to study stems, leaves, pollens, spores and many other botanical subjects. It must have been a great inspiration to observe, for instance, the minute spores of the fern or transparent pollen grains. These subjects are today still difficult to study and photograph, and it is quite impossible to see the spores while they are actually being thrown out of the sporangia of the fern.

In the year 1850 photography was in its infancy and the microscope was also a rudimentary instrument, the optical system being far from perfect. Considering the problems of photographic recording with the microscopes of those days, the results obtained were very creditable, particularly since only wet plates were available at that time. The introduction of dry emulsion vastly simplified photomicrography, which is now recognized as constituting a highly specialized branch of photography.

Great advances have been made in the design and construction of microscopes during the past few years and various types of photomicrographic cameras and microscopes are now specially constructed for photomicrography. These special models are to be found in industry, hospitals and research laboratories. In addition, the electron microscope has extended the field of microscopy. Nevertheless, very many microscopes like the one illustrated in Plate 4 (*upper*) are still in use in homes where photomicrography is a hobby; often such a microscope has been handed down from father to son. Many laboratory workers, also, are striving to produce photomicrographic records with the simplest equipment. For these and others about to explore the fascinating subject of photomicrography, this book is written with the hope that they may derive some benefit from its pages.

Photomicrography has become an important branch of applied science in industry, many problems being solved through this medium. A worker taking up photomicrography as a career must acquire a working knowledge of the microscope as well as a knowledge of photography, while the microscopist has only the photographic technicalities to learn. The photographer, on the other hand, has to master the optical system of the microscope and apply his photographic knowledge. Without a sound grasp of both microscopy and photography, good work cannot be produced.

During the past twenty years the properties of the various stains have been greatly improved. Staining a specimen correctly in no way damages any delicate structure in the subject chosen; on the contrary, it will assist in differentiating tissues.

The great progress which has been made in optics offers the photomicrographer special systems, which enable subjects not usually visible through the normal optical system to be seen clearly. With the development of these specially constructed optical accessories, the range of interesting photomicrographic subjects has become unlimited.

It is suggested that those about to take up this work should first devote some time to photographing small subjects which are too large to be photographed through the microscope, preferably immobile subjects such as large mounted specimens of flies, spiders, parasites, corals, small flowering plants, mosses, the delicate underside of leaves and insects' wings. Small moving subjects can then be dealt with, the valuable information gained when photographing 'stills' being used to advantage. Minute moving subjects present many problems to the photomicrographer, subjects such as vorticella being a good example.

Another problem which repeatedly confronts the photomicrographer is that of illumination, which is almost always carried out with artificial light. The proper control of illumination requires much practice and great patience before the many small pitfalls can be avoided.

Ragged, untidy, dirty specimens will not make the best photographic subjects. There are many important opportunities for exhibiting photomicrographs and naturally only those of the highest standard are suitable. Obviously there is scope for judges to deal with work submitted for exhibition purposes in the field of photomicrography. A scientific print should not be retouched or defaced in any way; the true record of the subject should be retained. A bad negative cannot produce a good print and over-enlargement is to be avoided. It should not be forgotten that the camera does not cover up failures caused through insufficient care being taken in the use of the microscope.

Although many colour processes are available today, there does not appear to be any marked progress in colour photomicrography. Obviously, only a few workers are taking advantage of this development, owing perhaps to its high cost.

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PHOTOMACROGRAPHY

THE fact that in the amateur world little is heard of this branch of photography, which is usually taken to include reproduction from actual size to ×20, suggests that photomacrography is not practised very much. In the professional scientific field, however, photomacrography is used quite extensively and proves invaluable.

An enlarged photograph of a minute object taken with a camera together with a microscope is known as a photomicrograph. An enlarged photograph of a small object taken without the aid of a microscope is called a photomacrograph. A microphotograph would be a minute picture and would require to be viewed through a microscope. It is most unfortunate that the term microphotography is often used in scientific literature when, in actual fact, reference is being made to a photomicrograph. Drawings made from images projected from a microscope are referred to as micrographs.

Photomacrography is less likely to confuse the pictorial worker than is photomicrography, as only one lens and an ordinary camera body are used. Plate 1 illustrates one means of obtaining a photomacrograph; other methods will be described later. Small objects with details barely visible to the naked eye require magnifying up to twenty times or so if they are to become of real value for record or scientific purposes. To obtain such magnifications it is not necessary to use a complex optical system such as that of the microscope. A simple camera lens suffices, and the details of opaque and transparent specimens may be obtained with reflected or transmitted light.

Photomacrography bridges the gap which exists between photomicrography and straightforward photography. Certain features involved in the design of the optical system of a microscope introduce some curvature of the field of view (Fig. 1). Unfortunately, an object lying in a flat plane may produce an image lying in a curved plane. Depending upon the degree of curvature of the image, it may not be possible to focus the central and peripheral zones of the image simultaneously. Therefore,

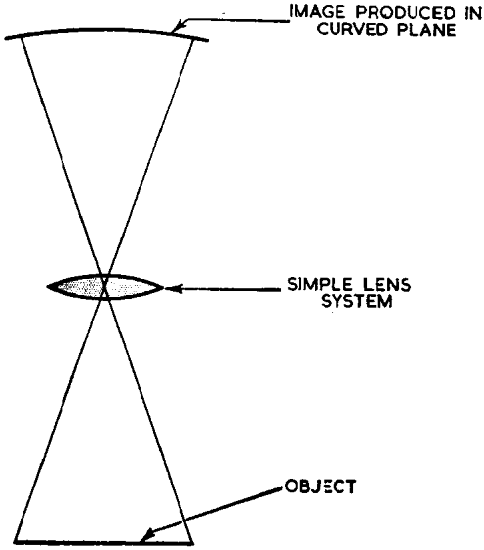

Fig. 1. Curvature of the image plane

when one photographs fairly large objects under the microscope, some difficulty arises in getting the whole field sharp. When one photographs subjects such as that shown in Plate 17, an instantaneous exposure is necessary, which means that the light must be bright and the emulsion–developer combination fast. These factors govern the lens aperture and shutter speed; even so, however, exposures may be surprisingly long. Electronic flash will permit the use of a much smaller aperture and very much shorter exposure, resulting in a far greater depth of field.

A selection of the many subjects which require photomacrography is shown in Plates 17 to 19, and 20 (*upper*).

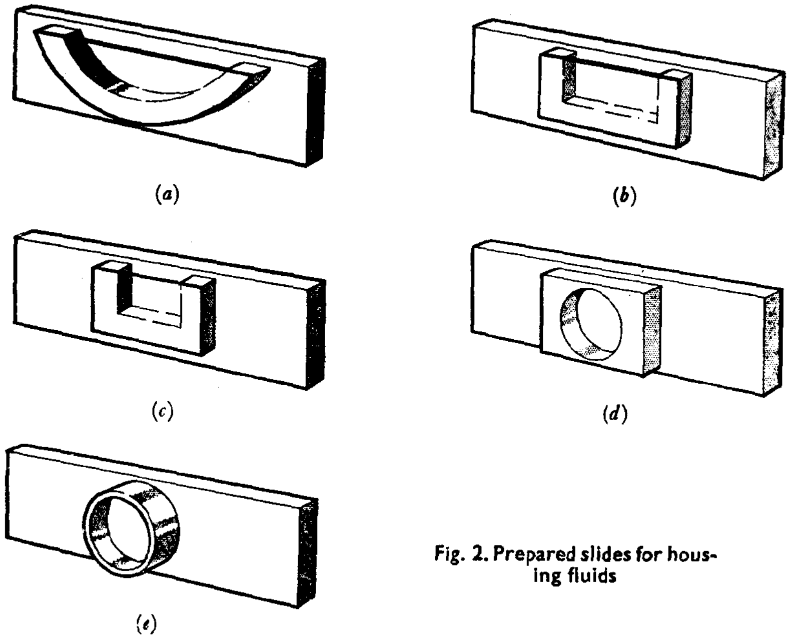

Fig. 2. Prepared slides for housing fluids

APPARATUS

In this branch of photography there are limitations as to the types of camera which can be used successfully. The most suitable is a plate camera equipped with treble bellows extension and having simple movements of lens panel and camera back. Both vertical and horizontal camera arrangements are suitable. The former is mostly favoured, especially for photographing tadpoles, static mounted and unmounted specimens, but there are some subjects, such as pond life, which cannot be recorded except in the horizontal (Plate 21 (*lower*)) and must be mounted in one of the first three slides shown in Fig. 2. This diagram shows a series of slides composed of fused microscope cells. Such slides are invaluable and can be used with either a vertical or horizontal camera arrangement. They are not cemented in any way, but are fused together, and will withstand normal heat and acids. Cameras such as the bellows type are best fitted with short-focus lenses, ciné-camera lenses or low-power objectives. If the camera does not have treble bellows, or even double bellows length, the requisite extension can be provided by bringing the lens forward by means of a metal tube, an improvised cardboard roll or even an extension box built on to the camera (Fig. 3). When possible, it is suggested that the camera lens should be reversed, that is, with the back lens facing the specimen. This position of the lens usually provides the best results since the optical conditions are similar to those for which they were designed. Specially constructed lenses for macro work should not, of course, be reversed. Focusing

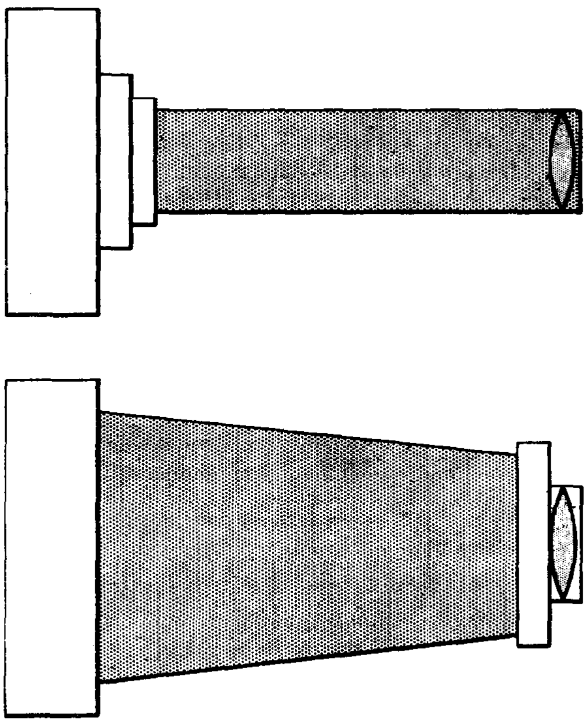

Fig. 3. Bellows and lens tube extensions

is carried out by raising and lowering the camera into its approximate position, after which fine focusing can be carried out in the normal manner. A ground-glass screen for accurate observation of the image is essential. By using a 2- or 3-in. lens it is possible to get within a few inches of the specimen but, of course, the depth of field is decreased accordingly. In view of this, a small aperture is needed to give greater depth; when small moving creatures are photographed, however, a small aperture becomes a handicap, as an instantaneous exposure is demanded.

The subject shown in Plate 17 proved to be very difficult to photograph, because working at such close range automatically created little depth of field, as can be seen by the out-of-focus area. Critical focusing on a certain point was carried out and a fairly large aperture used. Plate 20 (*lower*), however, was static, which enabled a long exposure to be given.

Since the magnification of the negative image is controlled by two factors, lens and camera bellows extension, it can be calculated by the following formula:

magnification $m = l - f/f$, where l is the lens-to-emulsion distance and f the focal length of the lens. Therefore, with a 3 in. lens and 21 in. bellows length, a magnification of $\times$ 7 is obtained.

To give additional magnification, an auxiliary lens can be used (see Fig. 4 and Plate 13). If the lens is uncorrected, difficulty will be experienced in filling the whole negative area without some detail falling away at the edges. Aplanatic magnifiers are, however, available. These are triplet lenses cemented

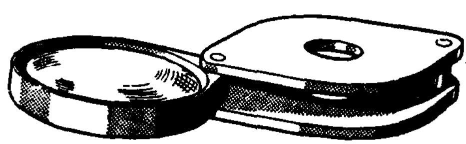

Fig. 4. Simple auxiliary pocket lens

together, giving a large flat field with excellent definition, and are superior to the single-lens magnifier.

Another method of giving extra magnification is the use of a converging spectacle lens of the meniscus type. A meniscus lens should be fitted in front of the existing lens, and a range of these lenses, which can be purchased quite cheaply from an optician, should, therefore, be to hand. The existing filterholder, or an improvised holder, can be used to house the lenses.

Reflex cameras, fitted with interchangeable lenses, are suited to this work, but the lenses must be fitted to a somewhat long tube to give greater magnification. The subject can be viewed in the reflex mirror right up to the time of exposure. With this type of camera particular care should be taken to ensure rigidity when the shutter is operated. When it is held in the hand, there is a tendency to move the camera as the shutter is being released and this must be avoided.

Twin-lens reflex cameras are not altogether suitable for this work, because two of everything must be procured, which entails additional expense. When using taking-lens and finder-lens as one apparatus, focusing is carried out in the usual way. To obtain an idea of the field covered with a Rolleiflex camera, the supplementary lens attachment can be fitted over the finder-lens, which is very effective in correcting the new field of view.

For miniature cameras such as the Leica, Exakta and Contax, there are interchangeable lenses and many other accessories for close-up photography. Spacing rings to extend the lens take the place of bellows and are very satisfactory. Some miniature cameras require an optical device for near focusing, while with others no difficulty is experienced, as the focusing mount of the lens remains the same and normal fine adjustment can be carried out. The greatest difficulty when using a miniature for photomacrography arises from the lack of a focusing screen, which makes it difficult to examine the whole of the image before the picture is taken.

The Aristophot II is ideal for this branch of photography, enabling translucent and opaque specimens to be photographed with the utmost precision and ease. Fairly large specimens, up to 3¾ in. in diameter, can be mounted and covered with the Summar or Milar series of lenses. The interchangeable stage, having insets with different apertures, can be selected to suit the particular object-field diameter. The specially designed lamphouse carries a stage for inserting the illuminating lenses and the specimen stage is adjusted vertically by rack and pinion. After the bellows length has been adjusted as required, focusing takes place by movement of the object stage. With objectives of short focal length, fine focusing is carried out by rack-and-pinion movement of the lens carrier. Final focusing can be adjusted by inspection of the image on the ground-glass screen. This particular macro-camera can also be equipped for use with transmitted and polarized light, and photographs may be taken with either the bellows camera or a Leica.

When the photomacrographic arrangement is used in incident light, the specimen is placed on the base plate of the Aristophot and illuminated by the ring illuminator. Adjustment is made vertically along the auxiliary bar attached to the base. The subject can be illuminated from all sides, or a cast shadow can easily be arranged by disengaging any one or more of the lamps. The ring illuminator consists of four lamps which can be adjusted and inclined at will. Alternatively, the 6-volt 5-amp lamp can be used.

ILLUMINATION

The recording of fine detail demands a great deal of skill in both vertical and oblique lighting and is of great importance. The lighting can either make or mar a picture. As the area to be photographed is small, it is more difficult to record detail; the light, therefore, must be arranged to make the best possible picture.

The shadows should help to tell the story when a vertical plan view is taken. Shadows of legs and body may be used to reveal an insect, which is normally camouflaged against the leaf upon which it rests. In a case such as this, therefore, the shadows need to be both dark and sharp.

There is no hard-and-fast rule as to the form of lighting to be used, but the 500-watt Photoflood lamps fitted into reflectors have many advantages. Lamps of lesser wattage can be used to good advantage, but the exposure time must be increased accordingly. Good results can be obtained by using one light in a reflector, together with a strong white sheet or card, which acts as a second reflector and lightens the shadows. Results free from shadow can be obtained by photographing the object on a piece of sheet glass acting as either an easel or a table-top. Various coloured backgrounds can be placed some distance from the glass, and these can be arranged to be out of focus, to leave the object standing out sharply against an evenly illuminated and shadow-free background.

With a working distance of only a few inches, it is essential to use a lens hood to prevent stray light from entering the lens. With small transparent specimens mounted on microscope slides, it is advisable to use a small microscope lamp or a × 3 or × 6 pocket lens, acting as a condenser. The slide may be placed about 6 in. above or in front of a sheet of white paper and the light-spot concentrated on the paper immediately beneath the slide. Great care must be taken to prevent glare or reflection from the glass cell and slide. On occasions it may not be possible to remove a specimen from its natural surroundings to the indoor equipment; in such instances there is no alternative but to make the best use of existing light. Shadows are accentuated in photomacrography and, in view of this, a diffused light is desirable. When insects are in a hibernating state, which usually means that they are in a dark corner, a pocket torch can safely be used, provided a sufficiently long exposure is given.

FILTERS

Care should be taken in the choice of filters (Plate 25). Money is always well spent on a set, and in the absence of a complete set one or two main colours are invaluable. When photographing insects and mounted stained specimens, a filter is almost always necessary to emphasize the part which is required. This re-introduces the problem of exposure, as the light absorbed by the filter necessitates an increase in exposure. Consideration must be given to the various colours of the subject in order to make it stand out from its background. Many specimens are double- or treble-stained, and in such cases it may be required to differentiate one particular tissue from the rest.

Let it be imagined that the section has been stained both green and red. If a red filter is used, red-stained tissues will become light in tone and the green-stained dark. Conversely, if a green filter is used, the green becomes light and the red dark. The point to remember is that a filter will lighten its own colour and at the same time darken the complementary colour to a degree. Filters can be either used between the light-source and the subject, if a lens system is employed, or placed at the back of the lens. In the former case the filter does not interfere with the sharpness of the image, but bad filters anywhere near the lens (back or front) can play havoc with sharpness.

SENSITIVE MATERIAL

As has already been implied, photomacrography is a scientific tool and the emulsion chosen will make or mar the final record, as in photomicrography. A first-class negative, which will produce a good print without any retouching, is absolutely essential. Retouching and print finishing is such a fine art today that the ultimate print can be a complete transformation of the original specimen; a scientific print, however, must be a true recording without any after-work, otherwise it ceases to be of value.

A hard-and-fast rule cannot be laid down as to the type of emulsion to be used, but certain recommendations can be made. Soft Gradation Panchromatic and P.1200 plates, or similar