GUIDE TO THE SCIENCE

OF

# PHOTO-MICROGRAPHY

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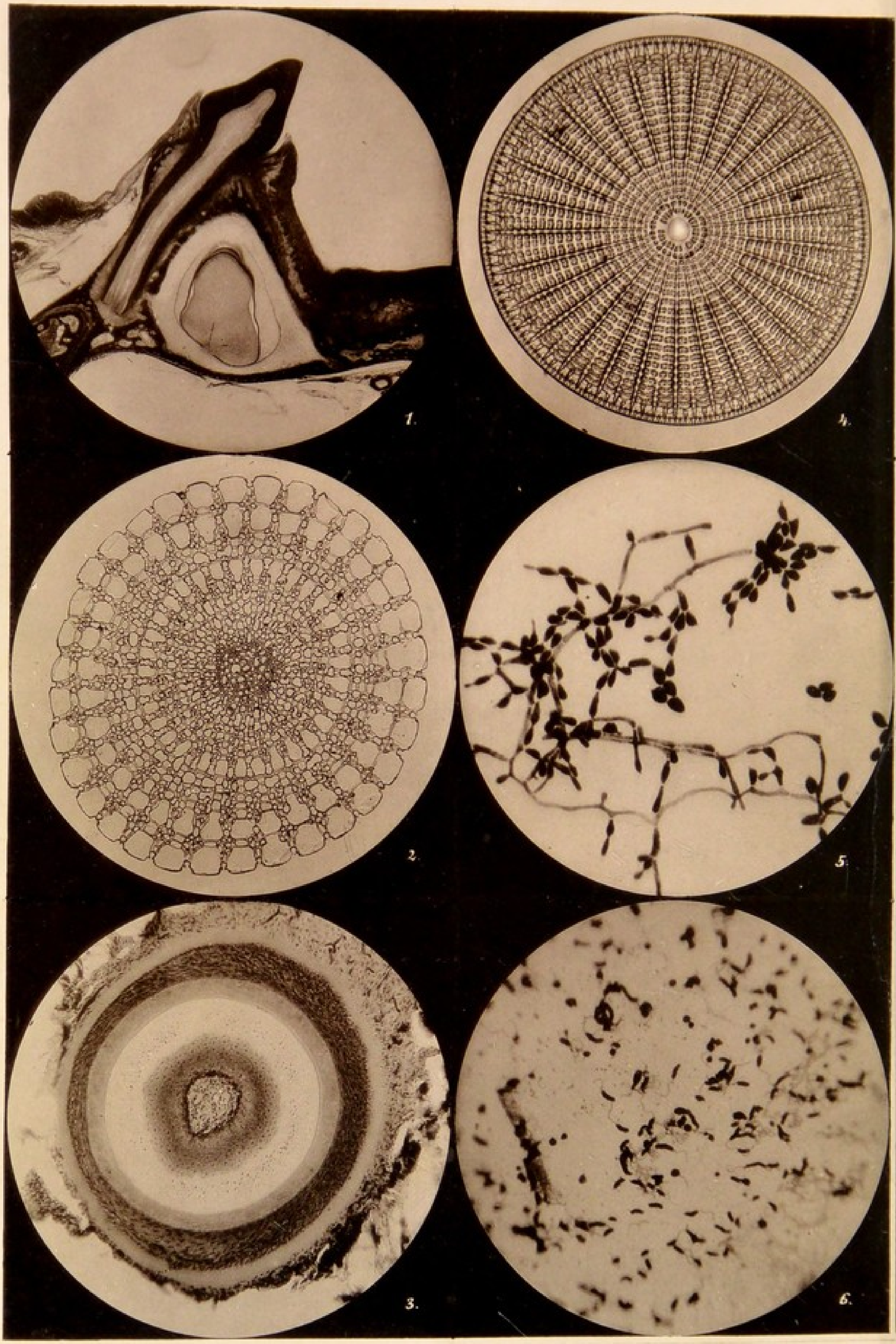

# GUIDE TO THE SCIENCE

OF

# PHOTO-MICROGRAPHY

BY

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L.R.C.P. LOND.

*SECOND EDITION*

ENTIRELY REWRITTEN, AND MUCH ENLARGED

LONDON

J. & A. CHURCHILL

11 NEW BURLINGTON STREET

1892

TO

DR. E. KLEIN, F.R.S.,

This work is Dedicated

BY HIS OBLIGED FRIEND AND PUPIL,

THE AUTHOR.

# PREFACE.

THE favour which has been accorded to the pamphlet which I published some years ago upon the subject of the present work, has encouraged me to rewrite and reissue it, embodying in it at the same time the results of a much wider experience.

My object throughout has been to show photo-micrography in the light of a handmaid in the service of observational science. For this reason, less space than is usual in such works will be found devoted to the representation of difficult diatom structure, and the name of *Amphipleura pellucida* occurs here for the only time, whilst stereoscopic photo-micrography is not referred to. On the other hand, a large amount of space is devoted to the consideration of the difficulties usually met with in histological and bacteriological work, and a new section has been introduced in which the method of photographing cultures is dealt with *in extenso*, whilst remarks upon the microtomic methods most suitable for the preparation of objects to be photographed are relegated to an Appendix.

If few acknowledgments of hints received from others are to be found in the following pages, this is due to the fact that, working almost entirely alone, the methods used have been the result of gradual evolution, rather than of absorption of others' ideas. Where I have consciously followed, the fact is mentioned in explicit terms.

It is to be hoped that this work may be found not less useful, on the grounds above mentioned, than its predecessor, by those for whom it is more especially intended.

It only remains to add that no attempt has been made to exhaust the photographic side of the subject. Those who wish to increase their knowledge in this direction cannot do better than master first the "Ilford Manual of Photography," by Mr. Bothamley, and then pass on to Messrs. Burton and Pringle's "Processes of Pure Photography."

EDWARD C. BOUSFIELD.

363 Old Kent Road, S.E.
*September*, 1892.

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NOTE.—Figs. 22-24, 28, 29, 31 and 32 are reproduced from the new edition of "Carpenter" by kind permission of the Publishers.

The frontispiece is from the writer's negatives, reproduced by Edkin & Co's "Glass-print" process.

# EXPOSURE TABLE.

| No. | Exp. | No. | Exp. | | No. | Exp. | | No. | Exp. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SEC. | | M. | S. | | M. | S. | | M. | S. |
| 58 | 1 | 49 | 0 | $12\frac{1}{2}$ | 40 | 2 | 45 | 31 | 42 | 40 |
| 57 | $1\frac{1}{3}$ | 48 | 0 | $16\frac{1}{2}$ | 39 | 4 | 20 | 30 | 56 | 0 |
| 56 | $1\frac{3}{4}$ | 47 | 0 | 22 | 38 | 5 | 45 | | H. | M. |
| 55 | $2\frac{1}{3}$ | 46 | 0 | 30 | 37 | 7 | 45 | 29 | 1 | 15 |
| 54 | 3 | 45 | 0 | 40 | 36 | 10 | 15 | 28 | 1 | 40 |
| 53 | 4 | 44 | 0 | 52 | 35 | 13 | 40 | 27 | 2 | 15 |
| 52 | $5\frac{1}{3}$ | 43 | 1 | 9 | 34 | 18 | 15 | 26 | 3 | 0 |
| 51 | 7 | 42 | 1 | 32 | 33 | 24 | 20 | 25 | 4 | 0 |
| 50 | $9\frac{1}{2}$ | 41 | 2 | 0 | 32 | 32 | 0 | 24 | 5 | 20 |

# PHOTO-MICROGRAPHY.

## CHAPTER I.

### PHOTO-MICROGRAPHY IN GENERAL.

History—Disadvantages of manual reproduction of images—Advantage of photo-micrographic reproduction—Limits of application of the latter—Attempts to render its use more exact—Distinguished workers at home and abroad.

The history of photography and that of photo-micrography are practically co-extensive. In the year 1802, Wedgwood and Davy, working in conjunction, produced the first photographic impressions of the images of external objects, and the impressions in question were those of the images of microscopic objects, projected by the solar microscope, and extremely suitable for reproduction by the very slow methods which these early workers were limited to.

These impressions were incapable of being permanently preserved, for want of a fixing agent, but the road thus opened has been traversed by thousands since, and every improvement in photographic *technique* has been applied in its turn to the purposes of photographic microscopy.

The Daguerreotype process yielded results, in some respects at least, of special excellence, and there are photo-micrographs still in existence executed many years ago by Draper, of New York, by Daguerre's process, which are of remarkable beauty, especially if we consider the limited means which the operator had at his command.

With the introduction of the collodion process, the cost of working was greatly diminished, and the number of workers thereby largely increased, but it is only within the last few years that any great advance can be fairly said to have been made upon the results of such workers as Dr. Woodward in America, whilst the advances have as a matter of fact been due, not so much to improvements in the methods of receiving the image, as to the great strides which have been made in the manufacture of the optical appliances by which the image is formed.

The introduction of the dry-plate, or gelatino-bromide process, with its greatly shortened exposures and its possibilities of artificial illumination, has certainly done more than anything else, from a photographic point of view, to popularise the practice of photo-micrography, and those who pursue this fascinating branch of microscopic science are under a deep debt of gratitude to Dr. R. L. Maddox, himself a photo-micrographer of no mean eminence, to whom is generally and, as the writer believes, correctly attributed the credit of the invention of the dry-plate.

Since its introduction the number of those workers who utilise photography as a means of recording the results of research, or reproducing the images of objects of interest, has increased enormously. Nor is this surprising. The older methods were both cumbersome and expensive, the time required for the execution of the necessary manipulations was very considerable, and the results upon the whole inferior to those producible by the average worker with the dry-plate, apart from the improved optical appliances now available.

The photo-micrographer of to-day finds himself in possession of easy, rapid, and satisfactory means of reproducing the image of almost any object, and in point of accuracy the results leave any method of artistic reproduction, through the medium of hand and eye, far behind.

Such artistic skill as that which made the name of the late Mr. Tuffen West deservedly famous is so rare that it may be left out of consideration. The average microscopist, if he has to depend upon his own skill, will certainly not be able to reduce his observations to a concrete form with anything approaching the speed and accuracy of even rough photo-micrographic processes. And even admitting that he possesses the needful skill for drawing the image he sees, such drawings are not, and cannot be, free from the "personal equation" which plays so important a part in the registration of scientific observations, and is one of the most fruitful sources of error in such work, whilst in any case there is always the risk that, beyond mere errors of observation, there may be the unconscious bias of the mind where the question of supporting or opposing a pre-existing theory is involved, and even the most skilful drawing may fail to render faithfully the details observed, from mere mechanical difficulties in the way of representation.

Allowing the possibility of the execution of the drawing, the question of reproduction must be admitted to have its importance, and here again, by any manual process, the same element of uncertainty is introduced.

From all these objections photo-micrography is free.

The image yielded by it represents one aspect at least of the object—the photographic negative will show nothing but what was transmitted by the lens, and that will be rendered with absolute fidelity, whilst the negative can be used to furnish an unlimited number of copies of the original image, with all its merits or faults.

The writer does not wish it to be supposed that he believes photo-micrography to be capable of doing anything and everything in the way of reproduction. Long practice in the art has made him only too well aware that there are objects which simply *cannot* be usefully or successfully photographed, and most probably never will be, at all events until some method has been devised for circumventing the existing code of optical laws. Difficulties in colour may, speaking generally, be always overcome, but there are a few objects whose form is such that no lens hitherto invented, or likely to be invented in the future, will ever be able to yield an image of them worth spending a plate upon.

Practice alone can be relied on to show the limits of application of the art. Difficulties which appeared insuperable at first will vanish as skill increases, but no skill and no lens will ever be likely to deal successfully with an object which is not at least approximately plane, and this applies very especially to objects which require the use of lenses of high aperture.

Photo-micrography cannot as yet be said to have risen far above the position of an art. Success in it has been, and probably always will be, due more to practical experience than to adherence to rules, to the application of previously acquired knowledge rather than to the carrying out of a scientific system of laws.

The first attempt to lay down an absolute rule for determining the most essential of all the elements of photographic success, the period of exposure, was made by Dr. Viallanes, in his pamphlet, published about five years ago.* Taking Warnerke's sensitometer screen as his standard, he laid down the following rule :—

The time required for the exposure of a plate is that which will suffice to impress the figure 13 of the screen upon the same plate under the same illumination.

Since the numbers of the sensitometer screen follow in regular order, each being one and one-third times as dense as the preceding one, the exposure of one plate in connection with the screen would enable the time of exposure of a colourless object, of fairly even density, to be accurately determined. With short exposures this plan might answer; with long ones it would involve very considerable trouble, and the present writer therefore endeavoured to determine what this length of time would be with a plate of any given sensibility, and a light of any given intensity, both these elements being determined as explained hereafter. (See chapter on Exposure.) A table, which embodies the results, and which was published in the previous edition of this work, is reproduced here in a more concise and correct form.

In concluding these preliminary notes, reference may be made to a few among the many who have more or less distinguished themselves in the practice of photo-micrography. The first place among English workers may be conceded to Mr. E. M. Nelson, not only on account of the extremely beautiful photographs of secondary diatom structure and of the most difficult

* "La Photographie appliquée aux Etudes microscopiques."

test-objects which he has produced, but because of his command of the optical principles involved in the production of the microscopic image and the generosity with which he has communicated the secrets of his success, and instruction in the manipulation of which he is so great a master.

Less well known, perhaps, but certainly not less successful in some of the highest departments of the work, is Mr. T. Comber, who has also produced photographs of diatom structure of the very highest excellence.

In general photo-micrographic work the writer has no hesitation in expressing his opinion that Mr. Andrew Pringle has certainly no superior. The character of his work is too well known to require any lengthened notice here, but his name will occur more than once in the following pages.

Dr. R. L. Maddox, who has already been mentioned in connection with the introduction of the gelatine dry-plate, is the *doyen* of photo-micrographers, and, had his health permitted, would scarcely have been passed in the race after excellence; but, though he has been compelled more or less to stand aside, his experience and stores of information have been ungrudgingly placed at the disposal of his fellow-workers.

Among recent additions to the ranks, Mr. Mummery has exhibited bacterial photographs of a very high degree of excellence.

Many other names might be mentioned; our English workers have but little to fear from foreign rivals. Born in England, photo-micrography seems to flourish most in the land of its birth, but its practice has spread to every civilised country, and the names of Woodward, Draper, Sternberg, and Mercer in America; of

Miquel, Viallanes, and Roux, in France; of Van Heurck and Neyt, in Belgium; of Koch, Fraenkel and Pfeiffer, and perhaps best of all, of Neuhauss, in Germany, are those of practitioners of this fascinating art who have made for themselves a wide reputation in connection with it.

Nor is their example ever likely to be without a host of followers. Bacteriology has sufficed alone to call into the ranks a whole crowd of workers, and in no branch of microscopic science are the advantages of the application of photographic methods more conspicuous. With the most ordinary precautions in making the negatives, all measurements can be more accurately made from the latter than from the objects themselves, since there is no parallactic difficulty; and a permanent record of appearances is almost instantaneously obtained.

The capabilities of photo-micrography in particular cases will be shown in future pages; the limitations have already been adverted to. The greater the skill and experience of the worker, the wider will the limits of successful working become. Practice will suggest methods, often as the outcome of failure, which will lead to the accomplishment of what had been deemed impossible, and the best advice which the writer can give to the beginner is, "Never throw away a negative, however bad, until it has clearly revealed to you the causes of its badness." Every exposure should, and may, give valuable information, and it is by storing up such information, and bringing it to bear upon subsequent efforts, that the tyro becomes the proficient.

## CHAPTER II.

### PHOTOGRAPHIC ACCESSORIES.

Dark room—Its illumination—Its water-supply—Daylight illumination—Its disadvantages—Dishes—Chemicals—Developers—Fixing bath—Clearing bath—Dry-plates—Films.

SINCE there is no difference, so far as the treatment of the exposed plate, and the subsequent processes, are concerned, between camera photography and photo-micrography, the chemicals and accessories required in the one case will be identical with those to be provided for the other.

The first essential is the dark room, or some efficient substitute for it, in which to conduct those operations which require the exclusion of actinic light. The writer's dark room, or closet, is about three feet square, and is lighted by a lantern containing a gas-burner, and glazed at the sides with dark-ruby glass, and in front with one thickness of ruby and one of dark-yellow glass. Both the latter are required in dealing with orthochromatic plates, but the ruby alone is sufficient for undyed plates, and the yellow alone for lantern slides and bromide paper, so that the panes are arranged to slide in their frames, allowing the light to pass through either or both at the pleasure of the operator. The products of combustion are carried off, by a chimney of asbestos paper, into the adjacent chimney-breast, free ventilation being thus secured and damage to the plates stored in the dark room being obviated.

The tap which controls the light is placed outside the lantern, so that a feeble light for the early stage of development, and an increased one for the later stages, are obtainable without trouble. The water supply is contained in a jug, and, where circumstances do not allow of the sink's being placed in direct communication with the drains, it will be found better to adopt this plan than to have a supply of running water, with the almost inevitable result of frequent overfilling of the waste-water receptacle, necessitating wading in the dark room. The sink, which is lead-lined, communicates with a bucket placed underneath, which is emptied as required, and is well painted inside. The dark room is situated in a recess in the photographing room, and is made light-tight by double curtains, the inner ones being of black twilled calico. Shelves are provided on either side for the reception of plates and bottles, and a drawer beneath the table is found convenient for containing bromide paper.

The above description may be found useful by those who, like the writer, have to make the most of a limited space; for those who have larger facilities a room with a good north light, and an abundant supply of water, will be found more convenient and agreeable. In such a room the window should be furnished with deep ruby and yellow slides as already described, and it will be found best to cover the glass with tissue paper, white or yellow, in order to moderate the light. At the same time it is probably more convenient, more agreeable, and safer to work by artificial light, which is always under control, than to trust to one which may at one moment be excessive, and shortly afterwards quite inadequate.

A few words may be said as to dishes. The material which the writer prefers to all others for general purposes is "Granitine," a kind of porcelain, but with the advantage over ordinary porcelain that the glaze does not crack or chip off after prolonged use, as that of ordinary porcelain does. This material is by far the most easily kept clean, glass alone being its equal in this respect. Papier-maché dishes are very light, and practically unbreakable, and, beyond a tendency to chip at the corners after a time, leave little to be desired. The writer has used ebonite dishes a good deal, but finds them very much more fragile than might have been expected. Glass must be made heavy if it is to stand the unavoidable wear and tear of every-day use, and except where transparency is desirable, as in the process of reducing negatives, has no advantage over porcelain, which is much lighter and stronger. Whichever material may be selected, it is very desirable to have sufficient dishes to allow of separate ones being reserved for pyrogallic and iron developers, and for hyposulphite of soda solution. Three two-ounce glass measures will be required, and these should be graduated, at their lower part at least, to half-drachms.

The list of chemicals required is a fairly long one, but there is nothing in it which can be considered superfluous.

Pyrogallic acid.
Meta-bisulphite of potass.
Hyposulphite of soda.
Bromide of potass.
Oxalate of potass.
Red prussiate of potass.
Carbonate of soda.
Borax.
Sulphate of iron.
Chloride of gold.

Bichloride of mercury.
Hydrochloric acid.
Alum.
Strong solution of ammonia, S.G. .880.
Citric acid.

To these may, if desired, be added hydroquinone, caustic soda, and sulphite of soda. The developer which the writer has for a long period been in the habit of using is essentially the one associated with the name of his friend Mr. Pringle, as his "1-2-3" developer. Each ounce of the solution as made up for use contains one grain of bromide of potass, two grains of pyrogallic acid (to which is added one grain of meta-bisulphite of potass), and three minims of strong solution of ammonia. Mr. Pringle uses 10 per cent. solutions, and makes up to the required bulk with water. The writer finds it more convenient in practice to make up stock solutions as follows:—

| | | |
|---|---|---|
| I. | Bromide of potass . . . . | 40 grains |
| | Distilled water . . . . | 5 ounces |
| II. | Pyrogallic acid . . . . | 80 grains |
| | Meta-bisulphite of potass . . | 40 „ |
| | Distilled water, to make . . | 10 ounces |
| III. | Strong solution of ammonia . . | 160 minims |
| | Distilled water, to make . . | 20 ounces |

To make the developing solution, one drachm of I. is mixed with two drachms of II. and two drachms of III., and sufficient water to make one ounce in all. A third drachm of III. is added subsequently, as will be described under the head of Development.

The only fault which the writer has ever found with this developer is that, when used for orthochromatic plates, the whites often become coated with a film of an iridescent green colour. This film may be easily removed by means to be indicated hereafter, or its formation may be prevented by substituting three grains of carbonate of soda (not bi-carbonate) for the three drops of strong ammonia prescribed above. One of the great advantages of the "1-2-3" developer is, however, thereby sacrificed—viz., that it indicates with almost absolute certainty, after twenty seconds' action on the plate, whether the exposure has been correct.

The writer does not recommend either ferrous oxalate or hydroquinone as a developer for photo-micrographic work. One of the chief beauties of a successful photo-micrograph consists in the whiteness of the ground upon which the object is displayed, and the use of either of the two last-mentioned developers renders its attainment much more difficult than is the case with a pyro-developed negative. The ground which the latter gives is very much less transparent to actinic rays (the visual density being equal) than is the case with either of the former, so that longer exposure (or longer development) is required to produce sufficient density in the bluish-black ground which they give, than is necessary with pyrogallic development. The prolongation of either exposure or development beyond what is needed to bring out the finer details tends to want of vigour in the negative, and makes the production of good bromide prints or lantern slides much more difficult.

The fixing solution is composed of—

| | |
|---|---|
| Hyposulphite of soda . . . | 3 ounces |
| Water . . . . . . . | 10 ,, |

It should be changed as often as it attains a brown colour, and, where much work is done, it is worth while to save the old solutions for the sake of the silver salts which they have taken up from the plates.

The clearing solution may either be made of—

| | | |
|---|---|---|
| Citric acid | . . . . . | 2 ounces |
| Saturated alum solution | . . . . | 1 pint |

or one drachm of hydrochloric acid may be substituted for the citric acid, producing a solution which is cheaper, and keeps better.

The dark room and utensils, with the developing, fixing, and clearing solutions, having been touched upon, we next come to consider the question of plates.

The makers of the "best and fastest plate in the market" are so numerous that to choose between their productions is a matter of very considerable difficulty. The writer has worked with a good many kinds, and has succeeded in getting good results from nearly all of them. Those which he at present uses are Thomas's for work with objects presenting no difficulty in point of colour, usually Thomas's "Landscape" or "Extra Rapid" plates according to the exposure likely to be required. For coloured objects Edwards' orthochromatic plates, of medium rapidity for work with oxy-hydrogen light, and instantaneous for photographing by lamp-light, will be found excellent. The Ilford Co. have lately taken up the manufacture of orthochromatic plates, which they produce at a very reasonable rate, and the writer recommends these for trial.

In photo-micrographic work, as in ordinary photo graphy, the tendency shown by the tyro is to adopt the fastest plates procurable. This is certainly a mistake, and it is only where long exposures would be inevitable with the slower plates that the very fast ones have anything to recommend them. Density is very much more easily obtained with a plate of moderate rapidity; the latitude of exposure is also greater, and the risk of fogging less.

Finally, the writer does not wish it to be supposed that he decides absolutely in favour of the makers mentioned. Familiarity with any given brand goes a long way towards the production of successful results, and, as already said, the writer has used many kinds with satisfaction, Edwards', Cadett's, Fry's, Wratten's, and Marion's amongst them.

The use of films in photo-micrography cannot be strongly recommended, but when negatives have to be reproduced by any collotype process, flexible films present some advantages. Several makers supply them, but the writer has used only two kinds, Edwards' and Obernetter's. He recommends the former, since the treatment is in every respect the same as that required by the plates of the same denomination by the same maker, whereas the latter need very special care in handling and must be freshly prepared to give good results. Hydroquinone development is to be preferred for negatives intended for reproduction, and must not be carried too far.

# CHAPTER III.

## PHOTO-MICROGRAPHIC CAMERAS.

Forms intended solely for photo-micrography—Zeiss's—Swift's—Transition form—Baker's—Observers' forms—Bousfield's—Details of its construction—Watson's student's—Use of room as camera—Vertical forms of apparatus—The writer's apparatus applicable in both positions—Pringle's—Zeiss's.

The recent developments in photo-micrography have led to the production of numerous forms of apparatus to be used in connection either with a special microscope or with any microscope, and, according to the relation of the instrument to the camera, all these forms may be divided into two classes.

The first class will include those forms in which the photo-micrograph, as such, is the object to be attained. The second class will contain those in which the photographic processes are meant to be subservient to the ready and accurate registration of the results of observations.

As a type of the first class we may take first the large and complex apparatus of Zeiss, certainly the most elaborate and expensive which has yet been constructed. It is shown in Fig. 1. The microscope and optical apparatus are here supported upon one table, and the camera upon a second, entirely separate. The first table carries the electric light, *C*; condensing system, *L*; alum-trough, *F*; parallelising lens, *H*;

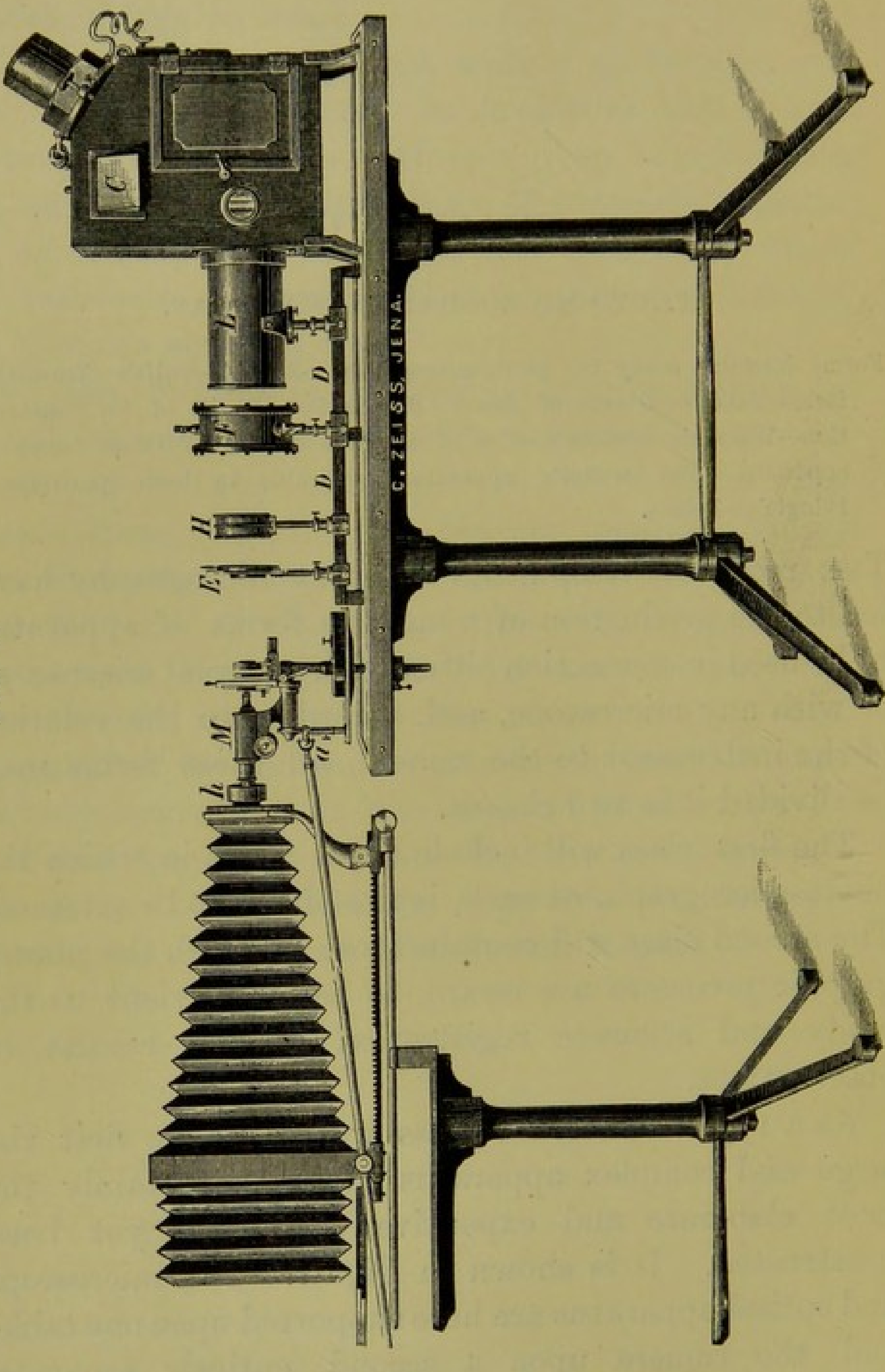
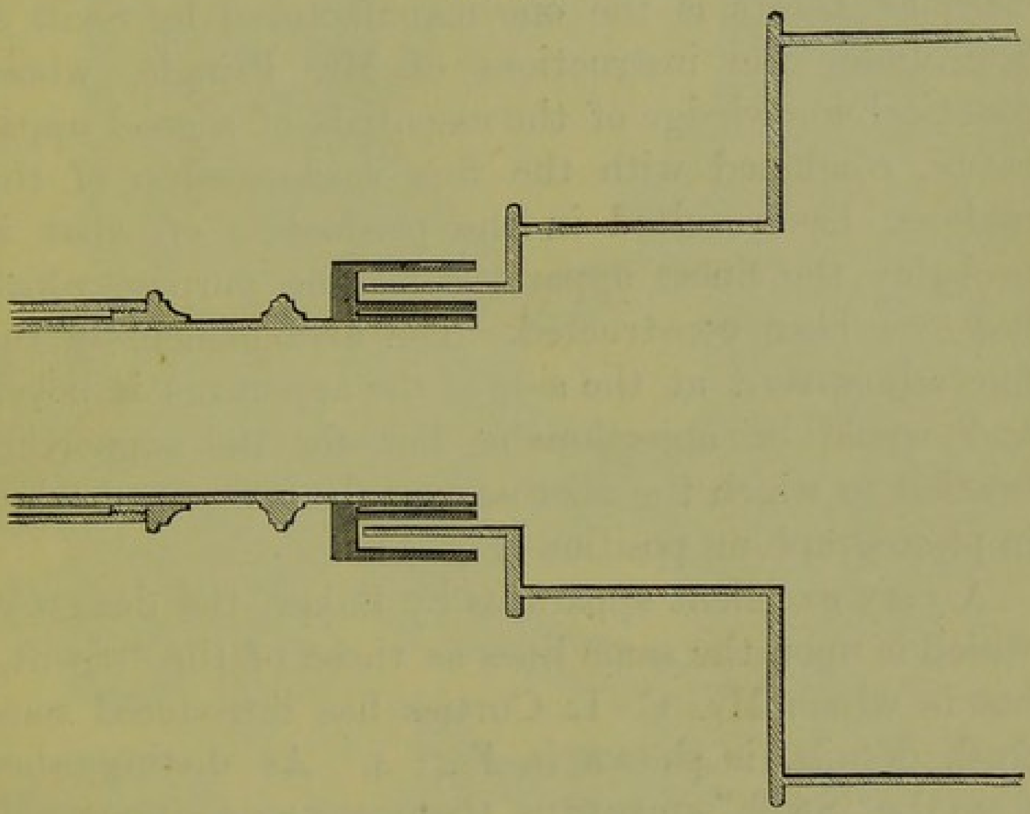



Fig. 1.

diaphragm, *E*; and microscope, *M*; all of these, save the microscope and lamp, being adjustable upon a horizontal bar, *D*. Upon the microscope is placed a light-excluding capsule, *h*, into which the nose of the camera fits, so that there is no contact between the camera and the microscope (Fig. 2).

FIG. 2.



The focussing is effected by a rod, with a "Hooke's" joint, *a*, which acts directly upon the fine adjustment-head, and this appears to the writer to be the weakest point in the apparatus. The direct transmission, without any arrangement for reducing the movement of the hand, is in itself an evil, but more objectionable even than this is the fact that the rod has to be taken up and laid down—a very dangerous proceeding where delicate work is involved.

It would of course be possible, though very inconvenient, to refrain from shifting the position of the focussing-rod, but even then the objection on the score of want of delicacy would remain, and in every respect the older arrangement, in which the connection was made by means of a worm-and-wheel gearing, must be considered far preferable.

An extremely fine form of apparatus in the same class as Zeiss's is the one manufactured by Swift & Son under the instructions of Mr. Pringle, whose practical knowledge of the essentials of a good apparatus, combined with the fine workmanship of the makers, has resulted in the production of what is probably the finest apparatus for the purpose which has ever been constructed. The arrangement of the fine adjustment at the side of the apparatus is novel, and would be objectionable but for the supporting bracket to which the microscope tube is clamped when in photographing position (Fig. 3).

A very excellent apparatus by Baker, the design of which is upon the same lines as those of the "Swift," but in which Mr. C. L. Curties has introduced some fresh details, is shown in Fig. 4. As distinguished from the "Swift" apparatus, the microscope is removable from the base-board, so that it is available for ordinary observation, but, as in the "Swift," the instrument is placed upon a board which is pivoted to the base-board proper, so that the final centring can be conveniently done by turning the upper board, or carrier, bringing the latter at right angles to the base-board, which has a stop to arrest the movement of the carrier when it is replaced in the photographic position. The arrangement of the fine adjustment is very good, the cord which moves the milled head being placed nearly at a

Fig. 3.

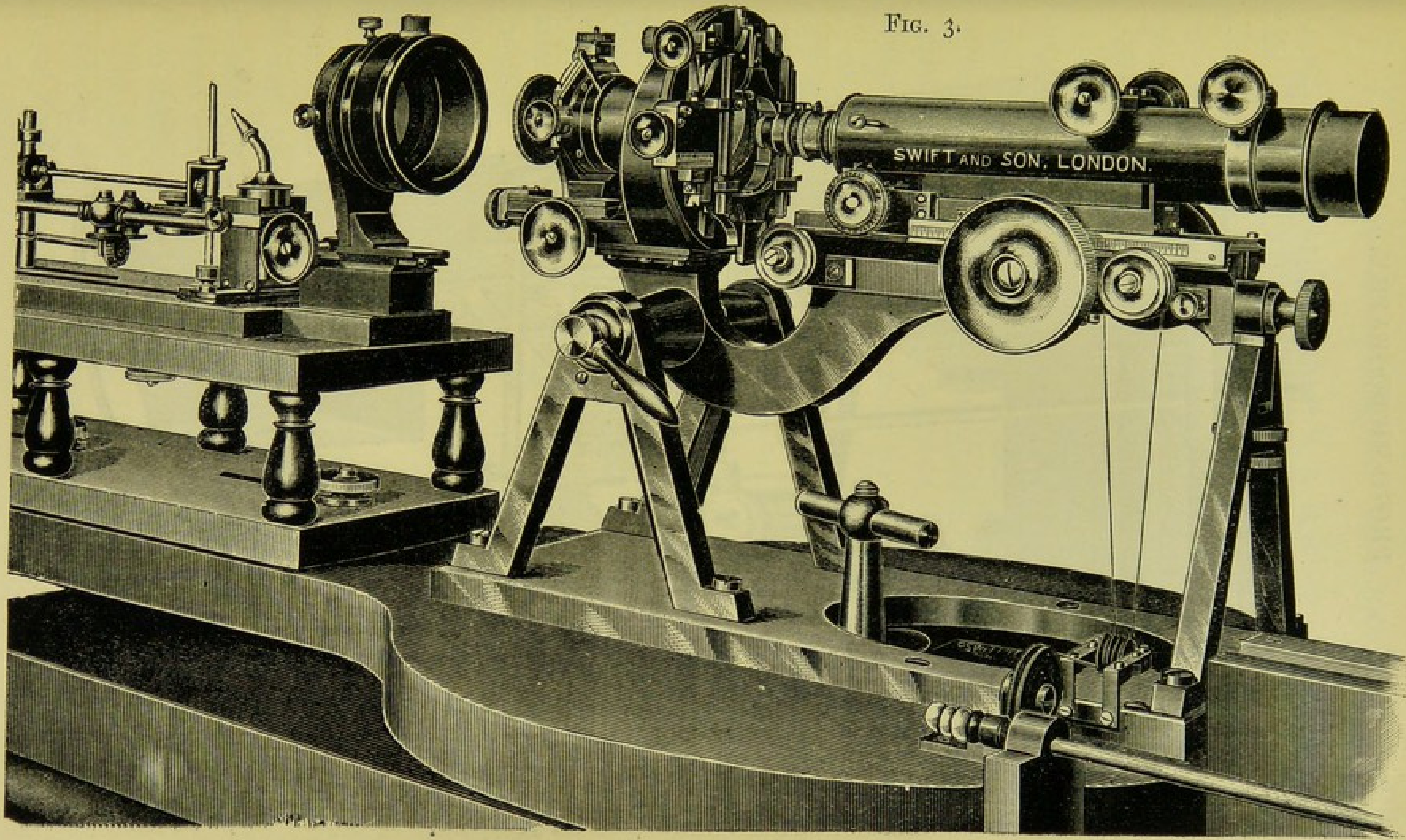
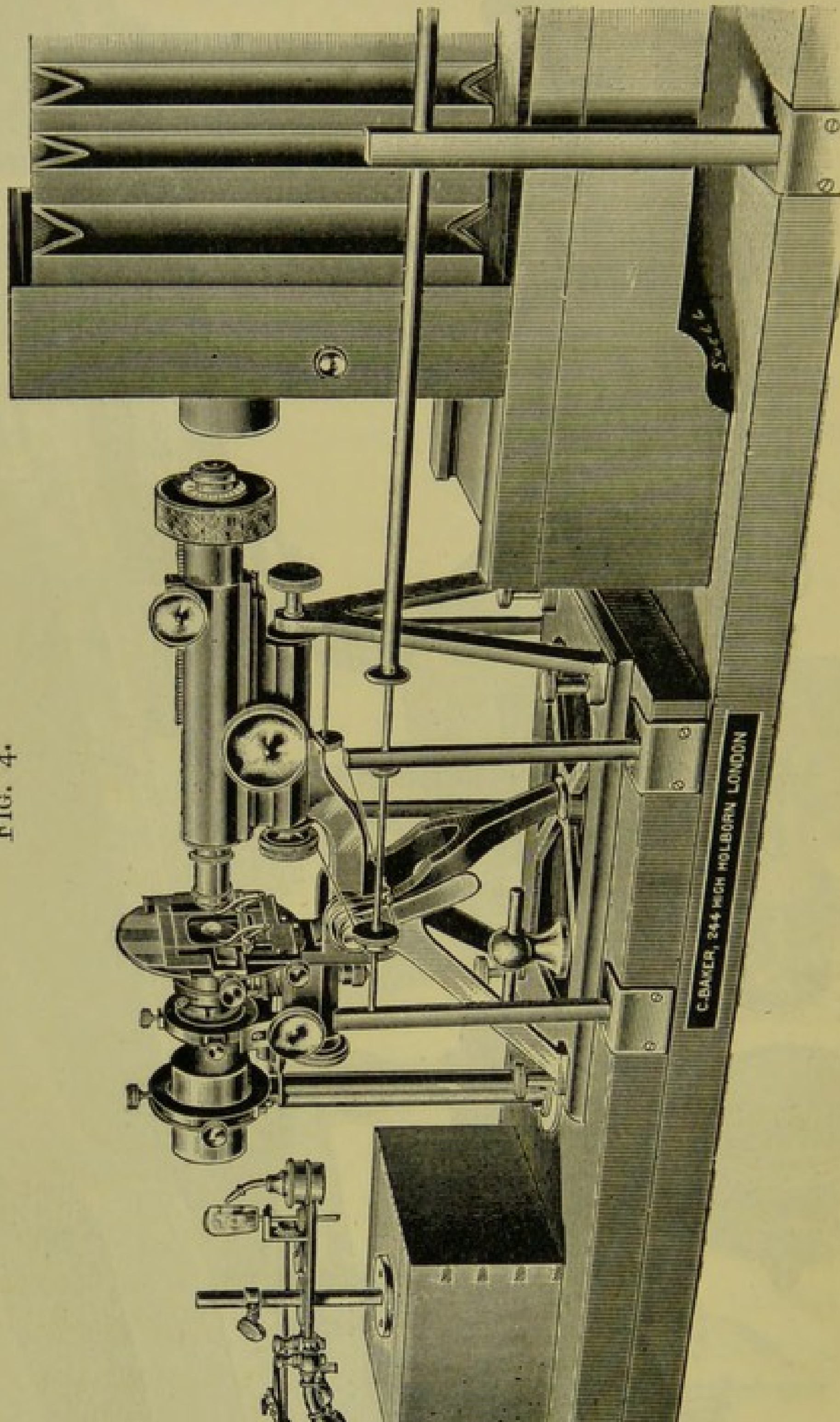


Fig. 4.



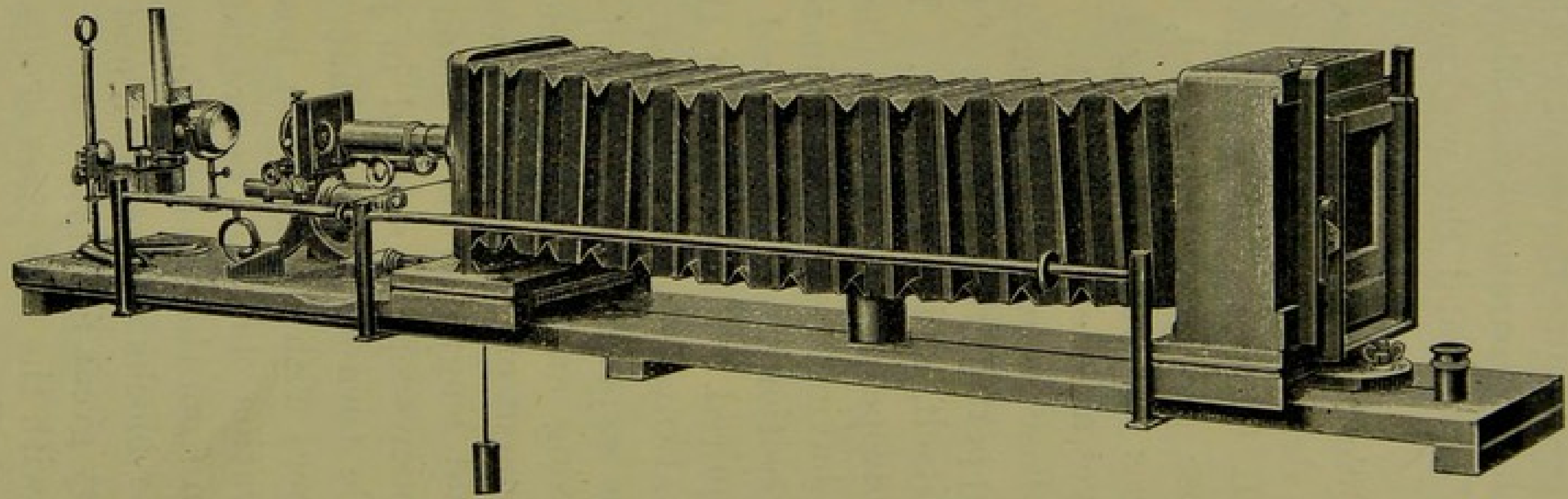
tangent to it, and, as there is a bracket in this case also for supporting the tube, there is little or no fear of any change of position as the result of the tension of the cord. The whole apparatus is considerably cheaper than the "Swift" form, and the workmanship is excellent.

The writer's own apparatus, also constructed for him from his own designs by Baker (under the supervision of Mr. C. L. Curties), differs in many respects from any of those previously described, and is the result of a process of evolution acting over several years. Fig. 5 gives a general view of it.

It consists of a base-board six feet long, upon which the carrier, the support of the nose-piece of the bellows, and the camera itself, slide in guides. The carrier receives the microscope and lamp (or other radiant), so that the two, having been once adjusted in their proper relative positions, can be moved along the base-board as required. The object of this is that the camera may always remain near the tail of the base-board, which experience shows to be the best position for focussing and other adjustments of the image. The movement of the carrier allows of an extension of the total length to about seven feet when required. The microscope is secured in position by blocks, with simple turn-buttons, so that it may be applied to the camera with the greatest possible ease. When an observation has to be registered, the microscope is taken from the laboratory into the studio, placed between the blocks and secured, and the negative made with very great facility.

The connection with the nose-piece of the camera is made by a light-excluding capsule (Fig. 2). The nose-piece itself is made to traverse in slides, vertically or

FIG. 5.



NOTE.—In order to show the traversing slides of the camera, the apparatus has been photographed at such an angle that the light-excluding capsule and the bracket which carries the pulley for the counterpoise are concealed by the support for the nose-piece of the camera. The pulleys shown upon the carrier below the milled-head are for the alternative arrangement described in the footnote on p. 24.

horizontally as may be required,* in a vertical support which runs upon guides on the base-board, and to the back of the upright is attached the bellows, which, in order to avoid internal reflection, is of considerable size —in fact, almost as large as the camera itself. The latter is made to receive a half-plate dark slide, but is about nine inches square, in order that there may be room for the action of a unique portion of the apparatus, the vertical and horizontal slides. The screen and dark slide are made to fit the vertical slide, so that they can be adjusted to the required point in height, and clamped there. The horizontal slide is then brought into action to secure the desired horizontal position, and also clamped.

The objects of this mechanism, which the writer has now used with much satisfaction for a long time, are two. Where only the centre of a field has to be represented it will be found by no means so easy as might be supposed to secure the coincidence of the centres of the field and the plate, especially in the case of a round object with a circular mask, where eccentricity is at least undesirable. The traversing-back allows this to be absolutely adjusted without the necessity of moving the object, which may be in a perfectly satisfactory position as regards sharpness and illumination. In the use of high powers another source of trouble arises from the fact that very often the slide or cover-glass, or both, are so curved that the sharpest

* This may seem an unnecessary refinement, as, indeed, it would be were the only purpose in view the connection of the camera with the microscope. As, however, the writer substitutes for the slide which carries the nose-piece a slide which carries an ordinary photographic lens when he photographs cultures, or copies, or enlarges, as will be described hereafter, the centring of the lens then becomes a matter of considerable importance, and it is very useful to be able to shift it when necessary.

point is not at the centre of the field, and this fault, which may have been but slightly, if at all, noticed in the direct examination, becomes very evident indeed when the image is projected, all faults being thereby exaggerated. Here again the traversing-back comes in serviceably, and, in fact, the writer rarely exposes a plate without having made use of it to some extent in arranging the position of the image.

The extended-focussing arrangement which the writer adopts is upon the principle of the counterpoise, the thread which works the milled head passing from the focussing-rod over pulleys at such a height that the thread forms a tangent to the milled head, and passes once round it, the *vis viva* being supplied by the weight of a small bucket of shot attached to the thread beyond the second pulley. By causing the thread to take a tangential direction, lateral strains upon the milled head are reduced to a minimum, since the tension of the thread is constant; and vertical strains are altogether avoided.*

The foregoing somewhat lengthy description is that of an apparatus designed to afford the greatest possible facility of application to the procuring of records of observations, combined with those refinements which experience has shown to be necessary for the most delicate work, such as the photography of flagella of bacteria. But, however complex may be the details of the apparatus, they will fail in accomplishing their

* For a long time the writer used and advocated an arrangement by which the motion of the rod, considerably reduced by pulleys, actuated by catgut bands, was transmitted to the milled head. The range of motion so obtained was of course far greater, but the tension and relaxation of opposite sides of the bands, to say nothing of their elasticity, gave rise to a good deal of trouble in very delicate work, and he therefore devised the above method, which is original so far as he is concerned, though probably used previously by others.

purpose unless precautions be taken to guard against the disturbing influence of vibration.

No good results in high-power work need be expected if the apparatus be not perfectly steady, and to secure this the base-board is supported on three feet, in the bottom of which are cut V-shaped grooves, which receive india-rubber cords, three-eighths of an inch in diameter. The cords are placed at equal distances upon a teak plank, six feet long and nearly two inches thick, and this again rests upon five india-rubber cubes, one at each corner and one in the centre. The whole stands upon a table, the legs of which are supported by cement pillars, isolated from the flooring of the studio (a cement floor would be still better). With the above arrangements the writer manages to work successfully in spite of the ordinary traffic of two thoroughfares within twenty feet of the studio, but a springless cart as it passes by will give rise to visible vibration of the image in spite of all, and the addition of an inch or more of felt beneath the legs of the table is necessary to overcome it. By the use of a sufficient amount of such material it will probably be found possible to eliminate this very troublesome factor in all cases, so as to obviate the necessity for resorting to suspension, or flotation, of the apparatus. The price of an apparatus as above described is not exorbitant—about £8, exclusive of the microscope.

For those whose work does not call for the employment of high powers or lenses of extremely wide aperture, a much simpler form might suffice, and such an one is shown in Fig. 6, which is an illustration of a students' apparatus by Watson, not faultless in its details, but which, with an amendment of the connection between the focussing-rod and the fine adjustment, would answer most purposes for magnifications up to three or four hundred diameters, and will yield quarter-plate negatives.

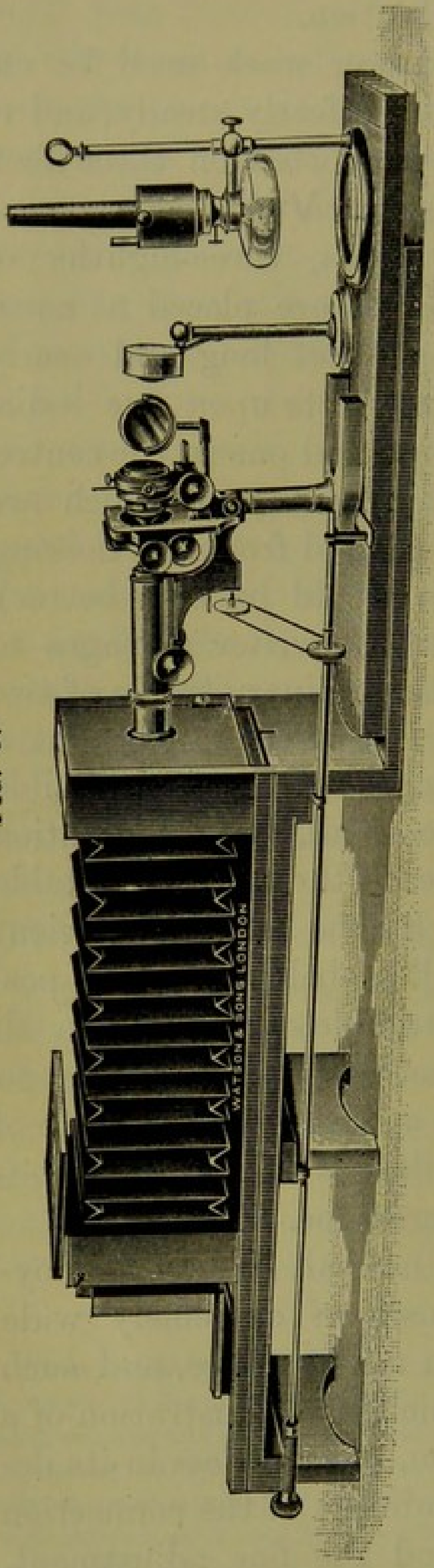



Fig. 6.

Simple as this camera is, it is yet in excess of absolutely indispensable requirements. An ordinary photographic camera, with the lens removed, and a tube built up of books and cloths, served the writer in many of his early experiments, even for instantaneous photographs of living worms by magnesium light, and after years of experience he is by no means ashamed of some of the results so produced.

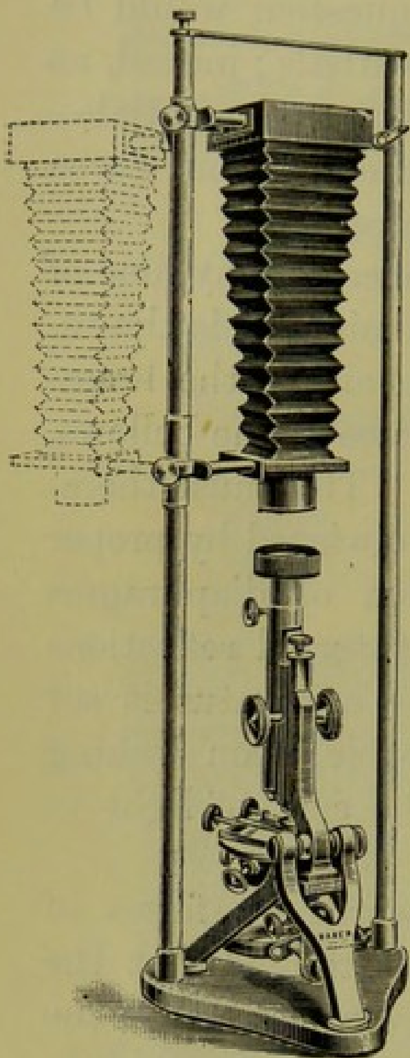
Again, where very large negatives have to be taken, it is by far the best plan to dispense with the camera (as such) altogether, and to project the microscopic image into a dark room, in which the plate is placed in a proper position for the reception of the image. Certain mechanical difficulties, as to focussing and so on, will no doubt present themselves, but can be overcome by a little ingenuity. Some of the finest work of earlier years, notably that of Dr. Woodward in America, was thus accomplished, so that further comment as to the merits of the method in question would be superfluous. It combines all the essentials; indeed, as regards the sensitive plate, there is but one essential—the exclusion from the plate of all the rays but those which pass directly through the microscope.

Of all causes of failure in photo-micrography, one of the most common, and most easily eliminated, is the reflection of light on to the plate from either the brass-work of the microscope, the inner surface of the bellows, or some portion of the camera itself. This must therefore be carefully looked out for, and obviated by proper blackening, and by the interposition of diaphragms at such points as to screen off the internal reflections from access to the plate. When the apparatus is set up for work, nothing should be visible, upon looking into the back of the camera, but the spot of light in the centre of the eyepiece.

It is necessary to add a few words on forms of apparatus in which the parts are so arranged that the stage of the microscope remains horizontal when in the photographic position. Some operators have a bias in favour of such a combination; a few are constrained by the exigencies of their work to adopt it; and in any case in which the objects to be photographed are so mounted that they are able to roll, it is unavoidable.

With the view of dealing with emergencies of this kind the writer's apparatus is so constructed that every part of it, except the radiant, can be clamped to the board, the relative positions being thus maintained whether the apparatus be horizontal or vertical. In the latter case it is supported against a wall, and insulated from the floor by a thick soft cushion; or secured, altogether off the floor, by holdfasts attached to the wall. In this case the light has to be reflected by the mirror, the heat ascending from the lime cylinder being too great to allow of the use of the oxy-hydrogen light, and the position rendering the use of any other direct radiant impossible.*

Fig. 7.



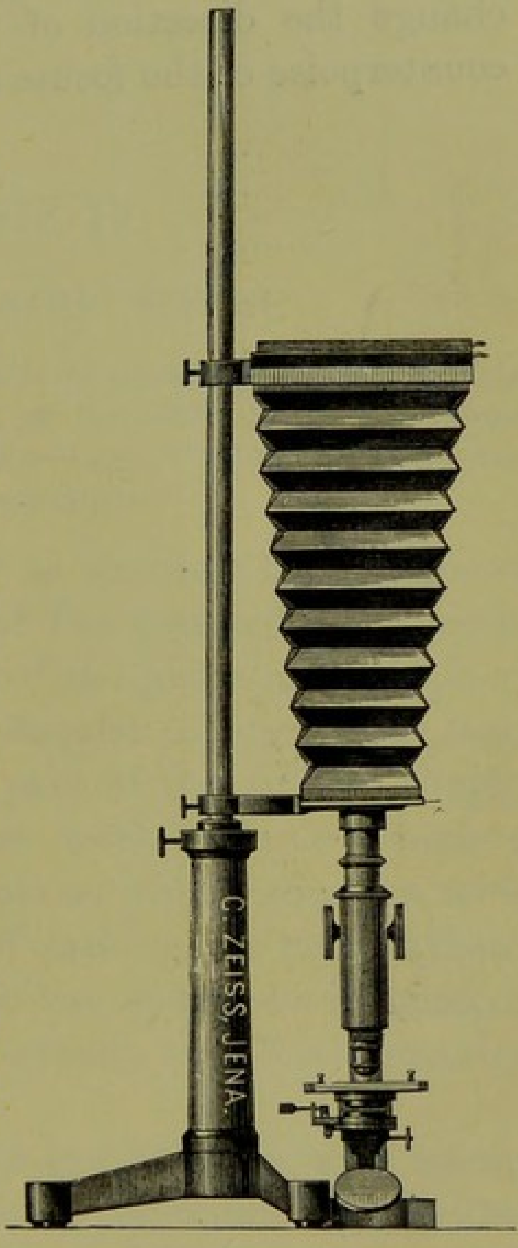
The previous remarks as to illumination apply to all forms of vertical apparatus, so that it will not be necessary to refer to the subject again in treating of them.

A very excellent and simple design is that shown in Fig. 7, the apparatus having been manufactured by Baker on lines suggested by Mr. Pringle. The special features of it are the ease with which the camera, as a whole, can be turned out of the way for direct observation, and restored to its position and clamped there firmly, when all is ready for the exposure. The range of

* This being the only case in which the use of the plane mirror is imperative for photo-micrographic work, one or two hints may be given as to its employment in this connection. One of the greatest objections to its use is that not only the silvered back surface, but the plain front one also, reflect the light, giving rise to a double image, and this being often distinctly separated into its two (often three) components, there are more illuminating cones than one. A very simple device, suggested to the writer many years ago by Mr. Nelson, suffices to obviate this. It will generally be found that the plane mirror is not tightly burnished into its setting, and if the image of the edge of the flame reflected by the mirror through the substage condenser

application is limited by the necessity for making the apparatus rigid, but there appears to be no reason why the same method should not be adopted in a larger instrument. All the movements are made with great facility, and the general workmanship is excellent.

Fig. 8.




Another very good apparatus is one made by Zeiss, and shown in Fig. 8. Here the camera is supported by a single rod, and the whole arrangement is much heavier than in the form last described. The writer is not prepared to say that this is an advantage; rigidity being secured, weight is only an encumbrance beyond a certain point.

Neither Mr. Pringle's nor Zeiss's apparatus is supplied with an extended-focussing arrangement. This is certainly a disadvantage, and must tend to limit the usefulness of both. In designing his own apparatus, the writer kept in view the desirability of its being capable

be examined with a one-inch objective, the multiple images may be made to coincide by simply rotating the glass of the mirror in its setting. A "first-surface" mirror, in which the reflection takes place from the front of the glass, or from the metal, is of course free from this defect, as is also a totally reflecting prism, which is a very excellent means of indirect illumination.

of performing the same work whether in the vertical or the horizontal position. The only changes made for the latter are the illumination by the mirror already described, and the arrangement of a third pulley to change the direction of the cord which carries the counterpoise of the focussing mechanism.

## CHAPTER IV.

### PHOTO-MICROGRAPHIC STANDS.

Complicated apparatus not indispensable to success—Mechanical *v.* plain stage—Essentials of stand—Form of foot—Fine adjustment—Coarse adjustment—Typical stands—Powell's—Large "Nelson" model—"Van Heurck" model—Zeiss model—Cheaper stands.

It seems scarcely necessary to devote a large amount of space to the discussion of the form of stand most appropriate for the purposes of the photo-micrographer. The latter is usually a microscopist to begin with, and takes up the photographic part of the subject simply as an adjunct to his other work. He is therefore likely to be provided with a stand which he knows, with which he is in the habit of making his observations, and which, even if faulty, he has learnt so to humour as to get out of it the best results that his ingenuity can compass.

It is surprising how much can be done with means apparently quite inadequate in the hands of a skilled and persevering worker. Some of the best work which the writer has ever seen was executed on a contemptible-looking little continental stand, the design and workmanship of which left everything to be desired, but the owner, being a man of skill, experience, and resource, managed, apparently with ease, to obtain results of the very first order. On the other hand, there are men possessing instruments of the highest class of workmanship, and with every accessory that money can purchase, whose results will never be worth a single year's interest on the amount they have expended, and who scarcely know the names, and certainly not the uses, of half the appliances they possess.

It is considered the proper thing, in a work of this kind, to show how money may be spent upon brass-work—and, indeed, the advantage of a really first-rate stand admits of no denial—but here, as elsewhere, the writer desires to impress upon his less experienced readers that there is more credit, and more satisfaction, in making good use of smaller advantages, and supplying the deficiencies by the exercise of skill, than in employing an apparatus of an almost automatic character, which will produce all work of average difficulty with almost the certainty of the "penny-in-the-slot" mechanism, and which has a mechanical appliance for every possible motion.

The delicacy of touch and facility of handling which mark the true microscopist are rarely to be found amongst those who have started with such an apparatus. A man who cannot, on a plain stage, move the object he is observing a ten-thousandth of an inch with absolute certainty, in any direction, needs to matriculate in microscopic manipulation.

For the benefit of those who contemplate the purchase of a stand, it may be advisable to enumerate the points which require attention under such circumstances.

The essentials of a microscope, apart from its optical portion, are two—a tube, and a means of adjusting it with reference to the position of the object. The tube itself must be rigid, or the lenses which it carries cannot both be upon the same optic axis, and here a very frequent source of trouble in high-power work has its origin. Again, the tube must be of sufficient width, and this the writer regards as one of the most serious objections to the continental pattern, in which the field is almost invariably cramped by the small diameter of the tube, and the consequent diminutive proportions of the ocular, whilst when the latter is dispensed with the fault is even more evident than before. Thirdly, for work of any special difficulty, the tube should be capable of being extended or shortened at will, in order to compensate for variations in thickness of cover-glass beyond the limits of correction of the objective.* With objectives of wide aperture, but without correction-collar, a variable tube-length becomes indispensable. Fourthly, the tube should be thoroughly blackened inside, or a disagreeable flare in the middle of the negative will call the worker's attention very decidedly to this point, perhaps at an inconvenient season.

As regards the stand itself, it may at once be said that the tripod foot, which gives three points of support (the least and the greatest number which confers absolute steadiness), is far preferable to the horseshoe, or any other form in which the weight of the microscope is supported by the whole extent of a single superficies; and the possessors of stands of the latter pattern are advised to have three studs inserted in the under-surface of the foot, in order, as far as possible, to secure the advantages of the tripod form. Even with this addition, narrowness of the base

* It is, of course, preferable to use only cover-glasses which are within these limits, but in constant working the expense and waste involved become a serious consideration.

gives rise to great want of steadiness, and the tripod form is in every way preferable.

The possibility of placing the microscope body in a horizontal position entails the necessity of putting a joint between the body of the instrument and the foot, and in the foreign stands this joint is usually placed below the stage, the result being that the body projects backward far beyond the foot, bringing the instrument into a condition of very unstable equilibrium, so that it becomes necessary to have a support beneath the tube near the eye-piece, a proceeding which is indeed worthy of adoption in all horizontal forms of apparatus, even in the case of the English model, in which, although the body is as a rule mounted on trunnions, and much better balanced, there is some risk of flexure of the tube when fully inclined.

The fine adjustment is a matter of very considerable importance, as success in delicate work will largely depend upon its steadiness and reliability, and to this fact the number of forms of mechanism which have been applied to the purpose probably owe their origin.

Into the details of the various forms it is not necessary to enter here, the points to be attended to are the smoothness, steadiness, regularity, and delicacy of the movement. One of the cheapest, and a perfectly reliable form, is the Campbell differential-screw slow motion, first adopted by Baker, and now largely employed by other opticians, with highly satisfactory results. It has the very great advantage that the whole tube is raised by it, and not simply an adapter at the lower end of the body, so that the tube-length, and therefore the magnification, remains constant during focussing, and the corrections of the objective are not upset. The same advantage is possessed by other slow motions, and indeed the old fashioned lever has nearly gone out of use, save in the very fine form of it adopted by Powell & Lealand, which has no superior.

One of the most serious faults in any fine adjustment is exhibited in a traversing motion of the images during focussing, due as a rule to curvature of the screw, the result being that the same portion of the object is not constantly in the centre of the field.

For ordinary work want of uniformity in the amount of "travel" at various points in the screw is not a very important point, but shifting in a longitudinal direction after the milled head has been released is fatal, if work with objectives of short focus be in question. It will thus appear that the two most important elements are straightness of action and staying power, and both must be carefully attended to.

The coarse adjustment, or quick motion, is a matter of less consequence. It may be effected either by the movement of the body through a jacket, or by a rack and pinion, and the choice between the two is usually determined by their relative cost, the former being of course the cheaper. In some cases, where objectives of very long focus have to be employed, a combination of the two is desirable and useful.

The relative advantages of plain and mechanical stages have already been alluded to. One thing is certain, that a bad mechanical stage is a delusion, and materially increases the cost of the stand without adding to the facility of working in proportion to the expenditure. A rotating stage may, on the other hand, be of distinct advantage, and whilst not necessary for the ordinary run of work, is very useful for that done with objects which are improved by proper orientation, and there are some which can scarcely be shown without it.

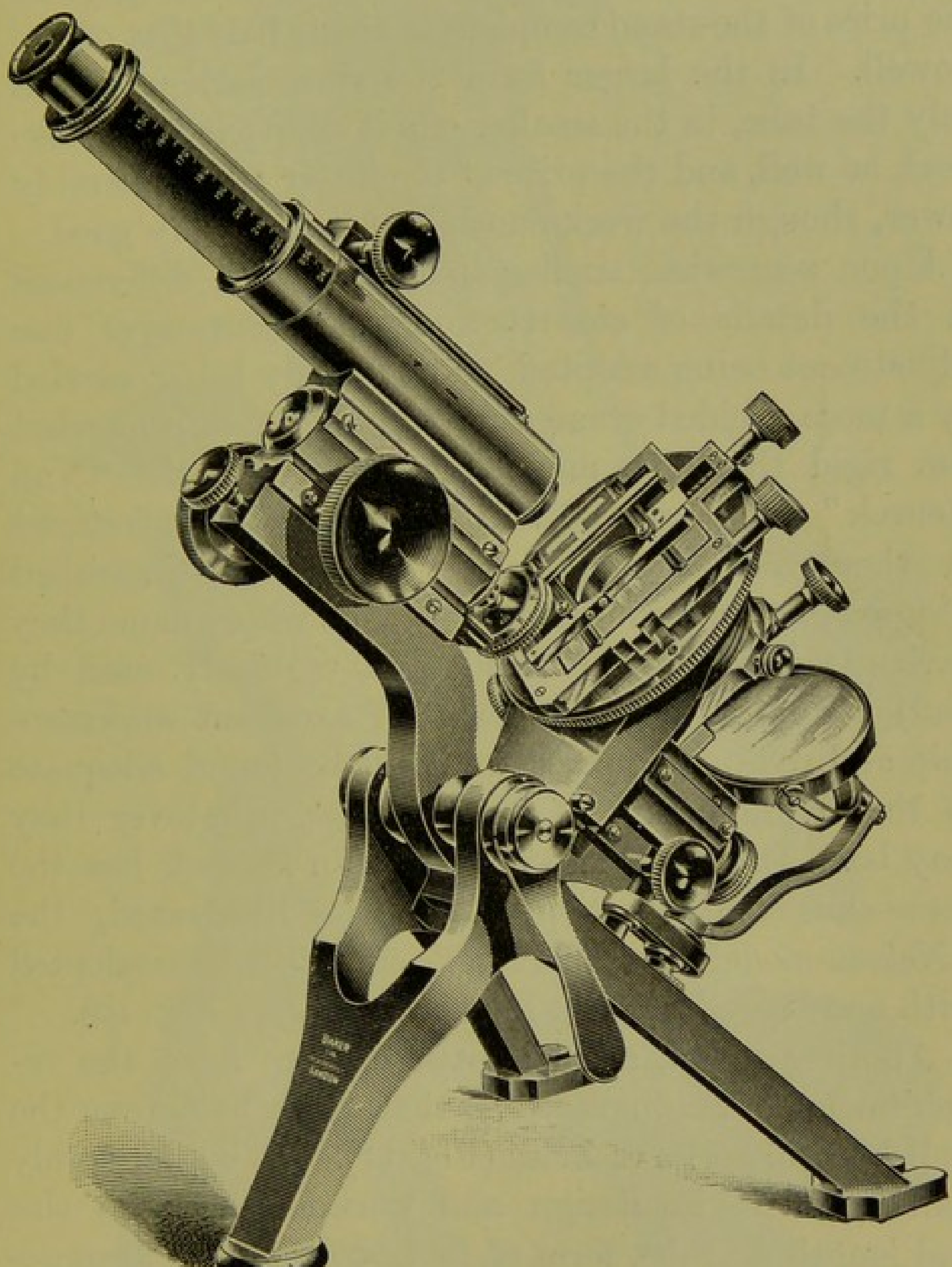
Having thus discussed generally the main features which require attention in stands for photo-micrographic purposes, we may now pass on to the consideration of a few type-forms.

The first place must be given to the larger stand of Powell & Lealand, which is too well known to require illustration here. Everything about it is as good as it can be, every detail of the workmanship has received the most minute attention, and *communi omnium consensu*, it is unrivalled, and represents the aristocracy among microscopes. On the other hand, the price is correspondingly high, the stand in question, without accessories, costing £50, and, fitted for the requirements of high-class photo-micrography, the sum to be expended could scarcely be reckoned at less than £120.

Following this prince of microscopes, at a reverential distance, are at least a dozen stands by various makers, three of which we select for illustration, in alphabetical order; one or two others, specially designed as photographic instruments, were noticed in connection with the apparatus which more properly belongs to that class.

A very fine stand indeed, equal to anything in the shape of work, and, upon the whole, preferred by the writer to any other form, is the "Nelson-model" instrument of Baker (Fig. 9). The structural lines of Powell's large stand are here avowedly followed, and this is the form which the writer would unhesitatingly choose, and to the possession of which at some future date he looks forward. Designed by one excellent photo-micrographer, and constructed under the supervision of another, the requirements of the art have been considered at every point, and yet the instrument is not

FIG. 9.



too delicate to stand the ordinary wear and tear of the laboratory. It has the body so constructed as to be adaptable to both long and short tube lenses, whilst additional power of extension is available for cover-glass correction if required; the fine adjustment is of the Campbell type, and is applied to body and sub-stage alike; there is an excellent mechanical stage, and the price of the stand complete is about half that of the Powell. In the larger form the slow motion carries only the tube, in the smaller one it supports the rack-work as well, and the price of the latter is considerably lower, though the workmanship of it is equally good.

Upon somewhat similar lines, but with differences in the details of construction, the Zentmayer fine adjustment being adopted, and the body being carried by a more decided crane-arm, and therefore somewhat less rigid than the model last described, the "Van Heurck" microscope of Watson & Sons (manufactured by them from a specification by the distinguished microscopist and photo-micrographer whose name they have attached to the stand, and regularly used by him) is a high-class instrument of excellent workmanship and reasonable price, and will be found adequate to the requirements of its possessor, whatever they may be. The instrument of Dr. Van Heurck has the horse-shoe foot; but in the form illustrated, the "Nelson-model" foot of Baker's stand has been adopted with corresponding gain in steadiness (see Fig. 10).

Another stand, specially designed to meet the requirements of photography, is that which hails from the well-known works of Zeiss (Fig. 11). It is thoroughly continental in its design and workmanship, the size and length of tube, form of foot, and general arrangements, being those characteristic of the foreign opticians. It is therefore an excellent representative of the class to which it belongs, having all the virtues of that class, and the faults being reduced to a minimum.

FIG. 10.

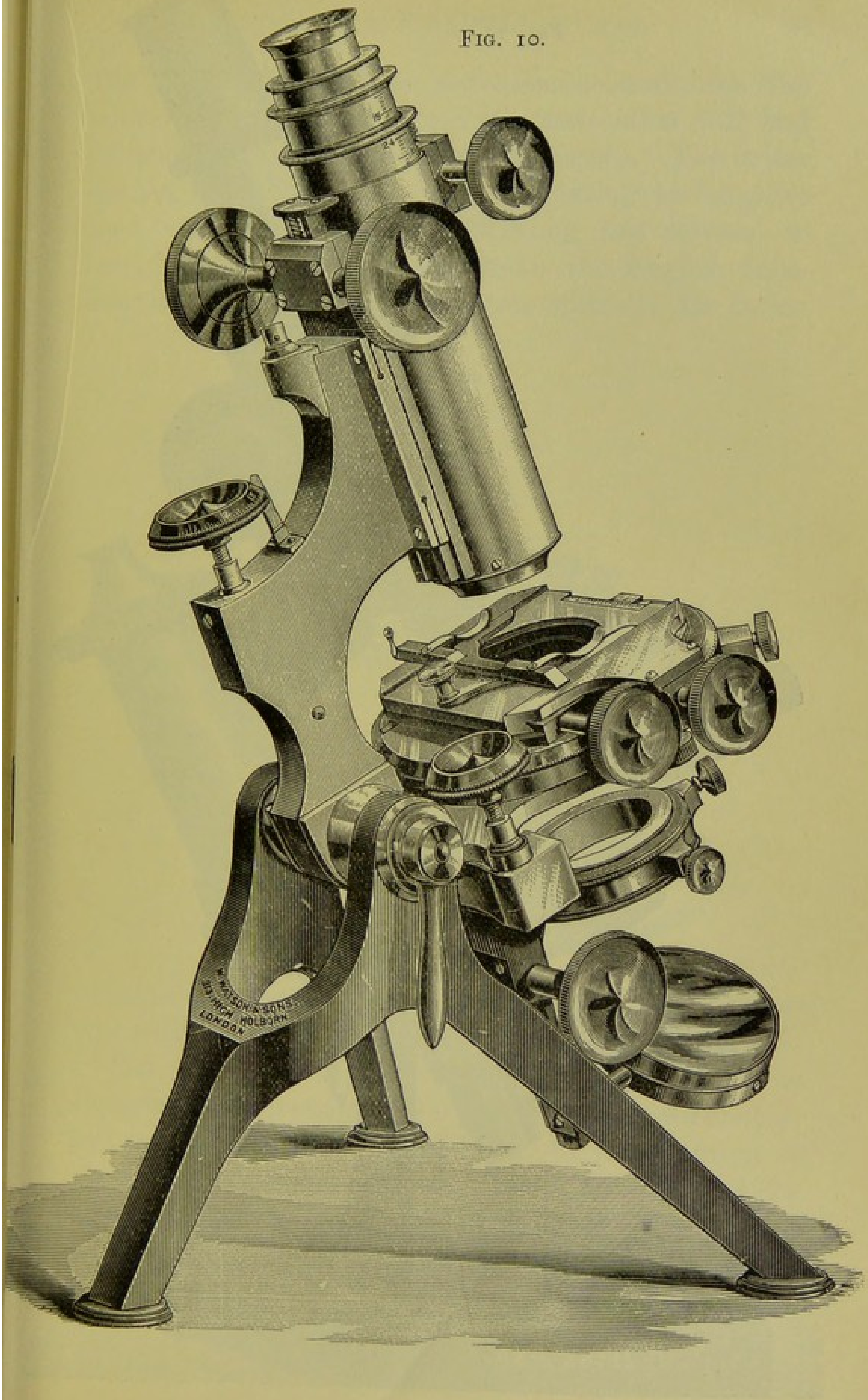
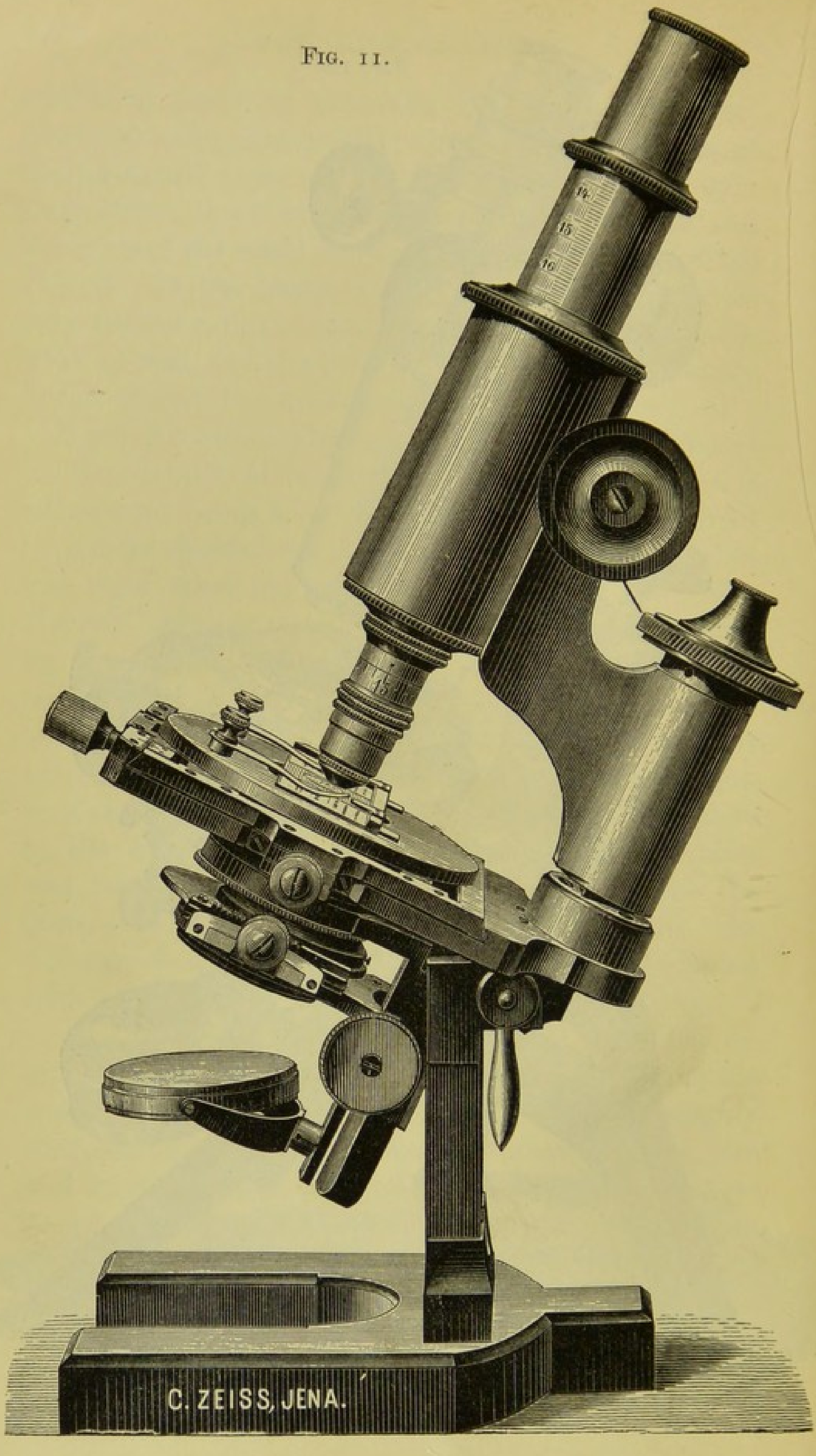
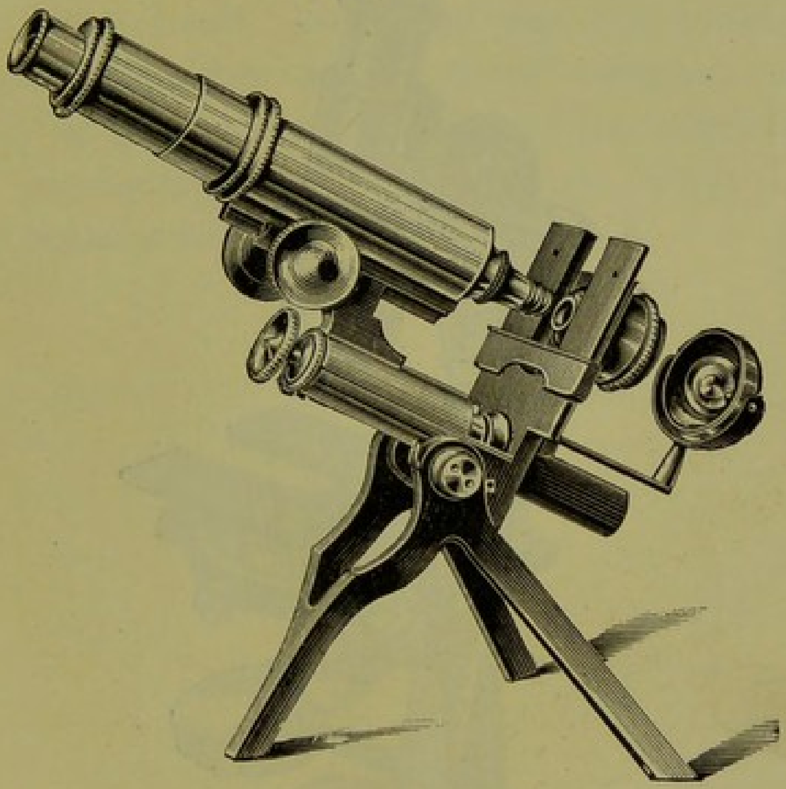


Fig. 11.



The price is high, but not unreasonable, and those who prefer this particular method of construction will find Zeiss's stand in every respect satisfactory. The writer cannot, however, refrain from expressing his surprise that whilst the Carl-Zeiss-Stiftung has striven to meet the tastes of those who prefer the English tube-length, by manufacturing lenses specially for it, no

FIG. 12.

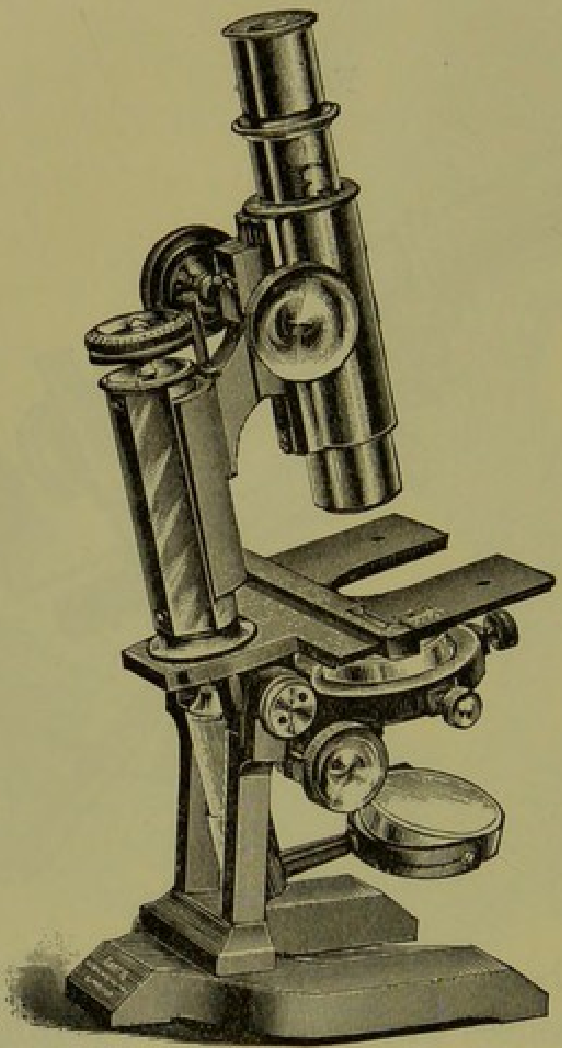


attempt has been made by it to supply stands upon which those lenses can be used to advantage.

The stands above referred to have been chosen as representative instruments of high class workmanship, but it must not be understood that by the mention of them it is intended to exclude those of other makers, of whom there are many, both in Europe and America, who supply microscopes which are capable of being used for any and every photo-micrographic purpose.

Swift, Ross, Beck, Walmsley, Tolles, Wales, Bulloch, Reichert, Leitz, Hartnack, and not a few others, will be able to furnish the intending photo-micrographer with what he requires in correspondence with his needs and means. In stands of cheaper construction,

FIG. 13.



the two forms illustrated in Figs. 12 and 13 may serve as examples of good workmanship applied to a simpler instrument than those previously referred to, the one having a tripod foot, the other a horse-shoe. They are both of Baker's manufacture.

It may, however, be questioned whether a microscope which is not furnished with focussing and centring arrangements for the sub-stage, will be found of any real service in the present condition of photo-micrographic work, and when these are added the price is of course correspondingly increased, to an extent dependent upon the particular arrangement adopted.

The writer's own stand cost originally £5, and is a Baker's "student's" stand, with large tube, but the substage condenser and fittings cost considerably more than the stand itself. Small as was the initial cost, the instrument, after seven years' constant use, is practically as good as ever, and does the whole of the writer's photographic and laboratory work to his complete satisfaction.

# CHAPTER V.

## OPTICAL APPARATUS.—I.

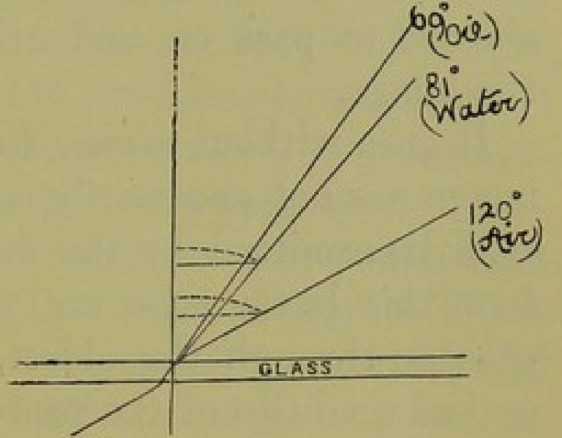
*Objectives*—Achromatic—Dry and immersion objectives—Angular aperture—Numerical aperture—Coincidence of visual and actinic foci—Apochromatic objectives—Their advantages—Zeiss's—Powell's—Roundness of their fields—Apochromatics by other makers—Achromatic objectives for photo-micrography—Tube length—English *v.* Continental—Objectives with and without correction-collar—Necessity of adapting aperture of condensor to that of objective.

THE question of the stand having been dealt with, we now pass on to those portions of the apparatus of the photo-micrographer which supply the *raison d'être* for all the others, and by which the whole of the work is actually done.

We shall deal with them in the order of their importance, and for that reason we take first the question of the objective, to which everything else is subordinate, and upon which the quality of the finished work, practically, altogether depends.

Two classes of objective will alone claim our attention—the achromatic and the apochromatic. These two classes are again subdivided into dry, water-immersion, and homogeneous-immersion objectives. In the case of immersion objectives, a drop of the fluid is placed upon the cover-glass, and fills up the whole interval between the latter and the front lens of the objective, so that the lens is *immersed* in the fluid, hence the name. The object of the immersion system will be understood at once by reference to the subjoined diagram (Fig. 14.) A cone of rays which passes from air into glass is so refracted on entering the latter medium that two things happen, the angle included between the bounding rays of the cone is narrowed, and, consequently, the point at which they would have previously met is removed to a correspondingly greater distance, in other words, the focus of the cone is considerably lengthened. The course of the rays in the reverse direction, however, depends upon the angle at which they strike the glass. Thus, any ray which is travelling in glass at an angle with the perpendicular to that surface of the glass at which it should emerge greater than 40°, does not get out at all, but is "totally reflected." If another plate of glass is placed upon the first one, this condition is not changed, since there is air between the two. If, however, a drop of water be placed between the two surfaces, the angle under which the rays may strike the surface and yet pass out is raised to 56°, and if a drop of cedar oil be taken instead of the water, there is no angle at which they will not pass out of the first plate, having once entered its lower surface.

FIG. 14.




Applying this principle to lenses, we find that, as shown in Fig. 14, the rays which in air form an angle of 120°, are compressed in passing through water to 82°, and in the case of cedar oil to 69°. It is easy to see how great is the gain in illuminating power on the one hand, and in working distance on the other, as the result of the immersion system, rays which have passed to the upper surface of the cover-glass at an angle exceeding the critical one between glass and air being allowed to pass on, and utilised for the formation of the image.

It goes without saying that the rays which form the image are not necessarily confined to the limits of the cone transmitted by the condenser. The rays which form this latter cone are variously refracted in their passage through the object, according to the varying optical qualities of the parts of which it is composed, and upon this fact, together with the absorption of some rays in their passage, the whole question of the formation of the image depends. The truth of this proposition would appear to be self-evident, and yet it is often lost sight of. It is absolutely proved, however, by the fact that if the condenser be of greater angular aperture than the objective, the whole of the dioptric beam may be cut out, so that no rays can pass directly to the objective, and yet an image of the object is seen upon a black background. This image is formed entirely by rays which could not have entered the objective at all had they passed through the object in a rectilinear direction from the condenser. Indeed, if this were the ordinary path of rays, no microscopic images would be possible in the case of colourless objects, except as the result of diffraction. This explains again the fact that, even with very inferior methods of illumination, there is a very decided advantage in the employment of lenses of high angular aperture, since the latter can receive and utilise rays which the object refracts to such an extent that they would be beyond the capacity of a lens of lower aperture. Before the days of immersion lenses the expression, "angular" aperture, sufficed to describe the resolving power of an objective. After the introduction of the immersion system it became necessary to modify the terminology, since we could only have expressed the aperture of a homogeneous-immersion lens by speaking of its "oil" angle, of a water-immersion lens one by its "water" angle, and of a dry lens by its "air" angle. All the difficulty and confusion which must have resulted has been obviated by Professor Abbe's introduction of the expression "numerical" aperture, and by multiplying the sine of *half* the angle of aperture in any given medium by the refractive index of that medium, we are enabled to refer to the apertures of all objectives in terms from which their respective resolving powers—*i.e.*, the number of lines to the inch which they are capable of separating, can readily be calculated.

The first requisite in a photo-micrographic objective is that it shall be so corrected as to have its visual and actinic foci at the same point. The chemical rays being more refrangible than the visual ones, special attention to this point is necessary, or else it is only by the very hazardous process of "taking shots" at the chemical focus, and exposing plates on an image which *cannot* be sharp to the eye, that a photograph can be attempted, though the difficulty may be obviated by the employment of monochromatic light, in which case all the rays, being of the same refrangibility, must necessarily be brought to a focus at the same point.

In the present state of microscopic optics there is little or no excuse for the employment of uncorrected lenses, and we do not propose to deal farther with this branch of the subject.

The question of correction for visual and actinic foci has little to do with those of achromatism and apochromatism.

Until a few years ago, the very best lenses, especially those of short focal distance, exhibited colour fringes round the object, which necessarily tended to a want of sharpness, a haziness of outline, or a disagreeable shadow round it, in the photographs taken with them. Only certain rays could be absolutely corrected for, the others being brought nearly, but not absolutely, to the same focus. This difficulty has now been eliminated. Aided by the liberality of the German Government, Messrs. Abbe, Schott, and Zeiss, working in conjunction, have produced various kinds of glass of such relative powers of refraction and dispersion, that by using them, in combination with fluor spar, in the construction of microscopic objectives, they have succeeded in practically abolishing colour in the microscopic image.* To lenses so corrected, the name "apochromatic" (which is colloquially shortened into "apo") has been applied, and their introduction has revolutionised microscopy and photo-micrography. Although the glasses obtained as the result of the experiments above referred to were placed upon the market, Dr. Zeiss maintained his lead in the manufacture of objectives from them for a very considerable period, especially as it was not generally known that fluor spar was an essential component in the combination of lenses required. Several makers,

* This has not, strictly speaking, been done in the objective alone, some portion of the correction being made in the compensation eye-pieces, to be hereafter referred to.

both English and foreign, followed in Zeiss's footsteps, but it is only quite recently that a success equalling his has been attained. The writer has lately had the opportunity of critically examining, and practically testing, a very fine homogeneous-immersion "twelfth," made by Mr. Thomas Powell, of the well known firm of Powell & Lealand (who kindly placed it at his disposal), and he has no hesitation in saying that it is in every way equal to the lens of the same focal length and numerical aperture, 1.4, of Dr. Zeiss, whilst in the very important particular of flatness of field it has a decided advantage. It is also provided with a correction-collar, and whilst this is much less important in the case of homogeneous-immersion lenses than in that of water-immersion or dry ones, it is an advantage not to be despised in delicate work, though a very considerable amount of experience is required for its full utilisation. In the matter of price there is a very great advantage on the side of the English lens, which costs £15 as against £25 for the German one of the same aperture.

Messrs. Swift & Son deserve great credit for the efforts which they have made in the same direction, and have produced some very good work, whilst on the continent Hartnack in Germany, and Reichert in Austria, have attained a very considerable measure of success. Much of Dr. Neuhauss's magnificent work has been produced with a Hartnack's apochromatic homogeneous-immersion objective of short focal length.

There can be no doubt that apochromatic lenses are the lenses of the day. Whether they will remain so has yet to be seen. It would be dangerous to say that finality has been reached in this branch of optical science. Indeed, Dr. Zeiss has produced an objective with a numerical aperture of 1.63, which, if it has done nothing else, has shown that the possibilities of "glass and brass" are not exhausted.

The writer confesses that he looks for future progress rather to improvements in mechanical methods, such as the possibility of grinding small lenses of parabolic form so that spherical aberration may be eliminated, than to advances based upon the use of media of higher refractive index.

Reference has been made to the flatness of field of the English lens above described (which lens, it should be said, is one of a whole series of apochromatics manufactured by Messrs. Powell & Lealand). The one objection to the apochromatic system is the roundness, the convexity, of the field of view, the result being that but a comparatively small portion, the centre, of the field is available for photographic reproduction. This can only be partially overcome, and it is a very serious drawback, since it is impossible to reproduce the image of an object which occupies more than about one-third of the diameter of the field of view, and even here we are getting perilously near the margin. The introducers of the system say that, in their opinion, nothing better is possible, Mr. Thomas Powell has shown that a little more can be done, and we may therefore yet hope that at least half the field may become available. If a three-inch disc, at a magnification eight times as great as the initial one of the objective in use, can be obtained, photo-micrographers will be well satisfied.* At present it is a matter of difficulty to obtain a lens which will give a disc of $2\frac{1}{4}$ inches diameter "sharp all over."

* Mr. Powell's $\frac{1}{12}$-inch lens just reached this.

The price of apochromatic lenses is high, and likely to remain so. They are, however, indispensable for the finest class of work. A "battery," comprising objectives of the respective focal lengths of 70, 24, 12, 6 and 2 millimetres, with the compensation-eyepieces, 2, 4, and 8, and a No. 3 projection-eyepiece, will cost £56 10*s*. or £61 10*s*., according to the numerical aperture selected for the 2 mm. objective. The writer's apochromatics are the 70, 16, 4 and 2 mm. (N.A. 1.3) lenses, and the cost of these, with the 2 and 8 compensation, and the No. 3 projection oculars, was £42. Still, in spite of the expense, he feels bound to say that he considers the money to have been well laid out.

Passing on next to the consideration of achromatic lenses, those of the ordinary type, we have no longer to deal with the productions of a few makers, since every optician of any repute supplies lenses of his own manufacture (or manufactured for him) which are of fairly good quality. As we are dealing with lenses solely for photographic microscopy the issues may be very much narrowed down, since only a comparatively small number of opticians make a specialty of objectives for this work. Thus Powell & Lealand, who stand admittedly at the head of the manufacturers of microscopic objectives in this country, and indeed are unrivalled as regards objectives of ordinary achromatic qualities, sacrifice the photographic powers of such objectives to the visual, so that the foci for the two purposes are not coincident.*

Baker, Swift, Watson, and Crouch, all supply objectives at a reasonable price, which will admirably fulfil the purposes of low-power photography, up to

* Their apochromatic objectives are here not in question; they have been spoken of above.

about 400 diameters, beyond this point there is a most decided advantage in favour of apochromatic objectives. The excellence of these latter has raised the standard of photo-micrographic work so much that it may be doubted whether any high-power work executed with other objectives would stand a chance in competition with that produced by the users of apochromatic lenses.

Zeiss's objectives must not be omitted in any remarks upon achromatic lenses. The writer has used many of them at various times, and has never yet found one which was wanting in coincidence of visual and actinic foci, and he still uses an *aa* (= about 1 inch) with very great satisfaction, it having remarkably crisp definition and a very flat field. In fact, he is unable to say that it shows any decided inferiority to the apochromatic of the same focal length, costing nearly five times as much, with the objects on which he has tried it.

Reichert supplies excellent objectives of the same class, but confines himself almost entirely to those corrected for the continental tube-length of 160 mm. Upon this point we shall have to say something hereafter; as to the character of the objectives themselves, it is very satisfactory. Reichert stands alone, so far as the writer is aware, in manufacturing semi-apochromatic objectives, which certainly appear to have some advantages over the simply achromatic form, though the writer's experience is not sufficient to enable him to speak with authority upon this point.

Other lenses and their makers might no doubt be referred to, but it appears unnecessary to enter at greater length into this part of the subject.

A few words on tube-length may be added here, since this is a factor of very considerable importance in the construction of objectives, and of paramount importance where those objectives are to be used for photo-micrographic purposes as well as for general observation. There are two lengths of tube in general use, the continental one of 160 mm., and the English one of 250 mm., corresponding respectively to 6½ and 10 inches (approximately) upon the English scale.

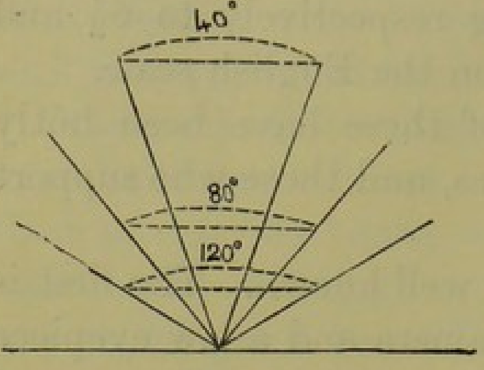
The relative advantages of these have been hotly debated, each has its advocates, and those who support the one denounce the other.

Two things are at any rate well known. The first is that it is better to use a long camera and a low eyepiece than a short camera and a high eyepiece. The second is that the lower the eyepiece, the more perfect and the better illuminated is the image. Putting these two together, it would seem clear that with a given "focal length" of the objective, it is *primâ facie* more desirable and more satisfactory to work with a long tube and a low eyepiece than with a short tube and a high eyepiece, to produce a given magnification. It is not possible, however, to dogmatise upon this point, since whilst Messrs. Nelson and Pringle, as well as the present writer, invariably use the 250 mm. tube, Mr. Comber, Dr. Neuhauss, and Drs. Fraenkel and Pfeiffer as invariably use the 160 mm. one.

One thing may at least be said, that long-tube objectives work very much better upon the short tube than do short-tube objectives upon the long tube.

In connection with the question of the choice of objectives, two things should be borne in mind. The first is that objectives of high aperture have a correspondingly shorter working distance than those of lower aperture, but of the same focal length—*i.e.*, magnifying power. The reason will be readily understood by reference to Fig. 15, where the plano-convex lens is intended to represent the system, and the angles are indicated by their proper signs. The respective "numerical apertures," for dry lenses, would in this case be .35 .64, and .87. The second point is that with lenses of high numerical aperture, both dry and water-immersion, the corrections are so delicate that slight variations in the thickness of the cover-glass interfere very seriously with the performance of the lenses, and unless a correction-collar be added, this can only be compensated for by alterations of tube-length which interfere very much with their normal standard of magnification. It is therefore necessary to keep as nearly as possible to the mean thickness of cover-glass for which they are corrected, and with the higher powers the correction-collar may be said to be indispensable.

Fig. 15.




With lenses upon the homogeneous-immersion principle, the index of refraction of the immersion-fluid is so nearly identical with that of the cover-glass, that the image-forming rays enter the objective in the same straight lines on which they started from the object, so that, with a given tube-length, no correction of these objectives is required. With water-immersion lenses, and as described above, with dry ones, the requisite correction has to be made either by means of a collar, or by altering the tube length. Every objective which has no collar is corrected for a fixed thickness of cover-glass, and the tube must be shortened for thicker covers and lengthened for thinner ones.

The need for correction is shown either by a want of sharpness in the image, or by the appearance of a mist, both being due to the fact that a portion of the rays has gone astray, with the result that they not only do not assist in forming the image, but by their effect upon the retina actually to some extent veil the image formed by the more parallel pencils passing through the central parts of the objective, which are far less affected by the variation in thickness than those pencils which are transmitted by the marginal portions, and since the excellence of any objective, and its defining power, depend upon the perfection of its marginal zone, it is evidently of great importance to utilise the latter as far as possible.

It is also evident that those misguided persons who persist in using pinhole stops in their condensers, or in trusting to the concave mirror for the illumination of lenses of wide angle, whilst they may, and do, secure immunity from "fog," are sacrificing almost the whole of the best part of their objectives, and really see little else than diffraction-images of their objects.*

* Makers are not free from the same fault. The writer has seen an excellent ½-inch objective so stopped down by the maker as to be almost worthless. Having removed the stop the writer was able to assure the purchaser that he was the owner of a very good lens indeed.

## CHAPTER VI.

### OPTICAL APPARATUS. II.—MICROMETERS.—FINDERS.

*Oculars*—The "pros and cons" of their employment—Projection-oculars—Their correction-collar—The method of obtaining a given magnification when using them—Compensation-oculars—*Condensers*—Principles of their employment—Sub-stage condensers—Use of bull's-eye as substitute—Concave mirror—Simple hemisphere—Abbe chromatic and achromatic condensers—Powell's dry and oil-immersion condensers—Faults universally present—Employment of objectives as condensers—*Micrometers*—General remarks on their character and employment—Maltwood's Finder—Method of using it—Writer's substitute for mechanical stage.

VERY much has been written for and against the use of the ocular—*i.e.*, the eyepiece, in conjunction with the objective, for photo-micrography. In referring to it here, one or two facts must be mentioned. There is no objective, except the 70 and 35 mm. ones of Zeiss, which is not primarily constructed for use with an eyepiece, and this is, in itself, a reason for supposing that, as such an arrangement gives the best visual image, it will also yield the best photographic one; there is also the fact that a very much shorter camera is required when an eyepiece is used, so that working is rendered more easy and convenient. On the other side there have to be considered the loss of light, and consequent increase of exposure, and the fact that, unless the eyepiece be one of low power, the image may suffer considerably in sharpness.

The writer has worked with and without, he does sometimes one and sometimes the other, and is guided chiefly by the size of the object, the magnification required, and the resolving power necessary to bring out the details of the subject.

The above remarks apply only to the use of achromatic objectives. When apochromatics are employed the case is altogether different. The corrections which ensure the total absence of coloured fringes in the image are distributed between the objective and eyepiece in such a way that the former cannot be made to yield its best results unless used in combination with the latter, and for photographic work a special eyepiece has to be employed for the projection of the image, which projection eyepiece is so constructed that the best results are obtained when the edge of its diaphragm is in focus upon the screen.

This point requires to be carefully attended to, and considerably complicates the process of obtaining any given magnification upon the screen, since every alteration of the correction-collar of the eyepiece necessitates a corresponding change in the position of the focussing screen. The one must therefore be carefully worked against the other until the desired result is obtained, the divisions of the micrometer being kept in focus whilst the sharpness of the shadow of the diaphragm is being arranged for. When high magnifications, with a correspondingly long camera, are involved, it is best to remove the camera altogether, and use a large piece of ground glass to obtain the exact magnification, afterwards replacing the camera so that its screen has the same image-distance as the ground glass had. The process is really not so troublesome as might appear, and if the results be noted down, as they should be, no trouble will be found in reproducing the desired conditions afterwards.

Thus, the writer's note-book contains the following remarks :—

500 dias. = 4 mm. obj., proj. oc. 3, at 5.5,* camera 24 in. long.

1000 dias. = 2 mm. obj., proj. oc. 3, at 5.5,* camera length 25.5 in.

333 dias. = 4 mm., proj. oc. 3, at 8,* camera length $15\frac{1}{4}$ in.

Similarly, a whole table may be constructed once for all, so that all ordinary magnifications may be obtained without the necessity of repeating the measurements. Where it is desired to produce such an image of any particular object as will fill a plate of a given size, the magnification must be measured after the photograph has been taken, as described hereafter.

One other point requires mention in this connection. The camera-length is always measured from the eye-piece-point, *i.e.*, from the point where the ocular rests upon the tube of the microscope, when projection oculars are used. Indeed all Zeiss's lenses, whether objectives or oculars, are so constructed that the absolute length of the tube has alone to be measured, whilst in the English system the tube-length is measured from the back lens of the objective to the diaphragm of the eyepiece, and thus varies, as regards its absolute position, with every combination of objective and ocular.

The compensation-oculars of Zeiss are not intended for photographic purposes, and cannot be so used at the visual focus, nor can the apochromatic objectives be used alone at that focus. In either case a correction

* This refers to the figures marked upon the correction-collar of the ocular.

must be made for difference of visual and actinic foci, and whilst Mr. Smith has produced some remarkable photographs of *Pleurosigma angulatum* by using the 6 mm. apochromatic objective and the "27" compensation-ocular, the plan is not one which could be recommended for general adoption.

The next optical appliance to be considered is the condenser, and inasmuch as this instrument is to the microscope what the slide-valve is to the steam-engine, its importance is only less than that of the objective and ocular in photo-micrographic work.

It has already been pointed out that the working qualities of an objective are in direct correspondence with the angular extent of the cone of rays which it is capable of admitting and utilising after their transmission through the object. The furnishing of this cone of rays is the purpose of the condenser.

From what has been stated above, it will be evident that the more nearly parallel are the rays which enter the objective the more will the formation of the image depend simply upon the loss of light consequent on the absorption of rays in passing through the object, that is to say, the more will the image come to represent a shadow, or silhouette of the object. It is thus that, where contrast is deficient in an object, it may be increased by cutting down the angle of the cone, so that this shadow-formation (and also interference phenomena caused by diffraction at the margins) may be called in to emphasize the outlines of the parts of the object which would otherwise be invisible. It is thus also clear that the less the cone has to be cut down the purer and sharper will the image be. A very large part of the skill of the photo-micrographer is shown in his power of balancing these two factors so as to produce the greatest amount of contrast with the least loss of sharpness.*

Of substage condensers† there are very many in the market, at prices varying from fifteen shillings to as many guineas, and it may be said at once that there is no single condenser which is available for all classes of work.

With very low powers it will be found requisite to dispense with the substage condenser altogether, and substitute for it a large bull's-eye, or other plano-convex or double-convex lens of long focus, in order to obtain the requisite evenness of illumination over a large area. Indeed, up to an angular aperture of 30°, either the bull's-eye or the concave mirror may be made to answer the purpose, and the latter has the advantage that it is achromatic, which is certainly not the case with the bull's-eye, in its ordinary form at any rate, whilst the achromatic bull's-eye has its focus so lengthened by the flint-glass meniscus that its use is rendered somewhat inconvenient, excellent as it undoubtedly is from other points of view.

All that is required is to so arrange the mirror that it casts a sharp image of the flame in the edge of the object, and the most satisfactory way is to find the radius of curvature of the mirror‡ and place the latter at the

* The contrast here spoken of is that of the object with the ground, and not of the parts of the object with one another, which will be dealt with in a subsequent chapter.

† Where the word condenser is used alone in this work, it must always be understood to refer to the substage condenser, the "bull's-eye" condenser being simply spoken of as *the bull's-eye*.

‡ This is easily done by setting a deep cardboard box (a dry-plate box will do well) by the side of the lamp, and then moving the mirror backwards or forwards until a sharp image of the flame is formed on the bottom of the box, *i.e.*, in the plane of the flame. The distance of the flame and box from the mirror gives the radius of curvature of the latter and the "principal focus" is half the radius.

length of this radius from the lamp and object alike, setting the lamp nearly in a line with the plane of the stage if possible, to avoid loss of light, though this is not essential. The angle of the cone of rays in this case will of course be equal to the aperture of the mirror (that is, to the portion of the whole circle which its curve includes), and this angle may be increased by placing the mirror nearer to the object and the lamp farther from the mirror or diminished by reversing the arrangement.

In dealing with a simple condenser, such as the bull's-eye, or indeed with any condenser, the same rule holds good, and this is a matter of great usefulness in practice, since, with a high-power condenser of short focal length and an object mounted upon a thick slip, or in a cell, the working distance of the condenser may be considerably increased by simply moving the lamp closer to it, though some aperture is thereby sacrificed.

The bull's-eye alone has about the same range of usefulness as the concave mirror, but will, no doubt, as a rule, be preferred to the latter, since it is much more handy to work with, and the chromatic aberration is not of great consequence in the low power work which will generally be done with this appliance alone.

Objectives of more than 30° angular aperture require a special substage condenser, and a few typical forms of this auxiliary must now be referred to.

The simplest of all is the hemispherical lens of about $\frac{4}{10}$ in. focus, which, mounted in a sliding fitting to adapt it to the usual substage jacket, and provided with a set of annular and central stops, may be obtained for about fifteen shillings, and, when properly used, is capable of accomplishing very great things, in spite of the spherical and chromatic aberration which are inseparable from its form.

Next in order may be placed the non-achromatic condensers of Abbe, manufactured by Zeiss at a very reasonable rate (Fig. 16). Though to a great extent superseded by the achromatic condenser designed and manufactured by the above workers respectively, these condensers are still largely used, in consequence of their high aperture and low cost. The Abbe-Zeiss achromatic condenser (Fig. 17) is undoubtedly the very finest apparatus of the kind for general photo-micrographic work. It has an aperture in air of nearly N.A. 1, and the iris diaphragm with which it is provided is placed between the lenses, as it should be. A very excellent condenser (Fig. 18), of about the same aperture, but higher in price, is manufactured by Powell & Lealand, on a formula of their own, and is probably freer from spherical and chromatic aberration than even the Abbe-Zeiss form. The latter has been copied, it may be remarked, by several English opticians (with varying degrees of success); probably the productions of

Fig. 16.

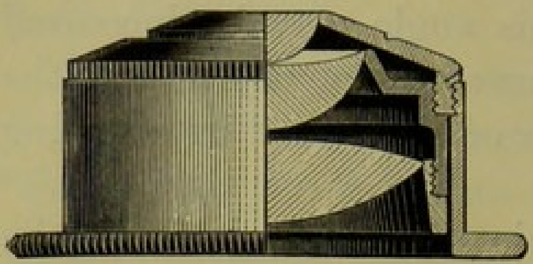
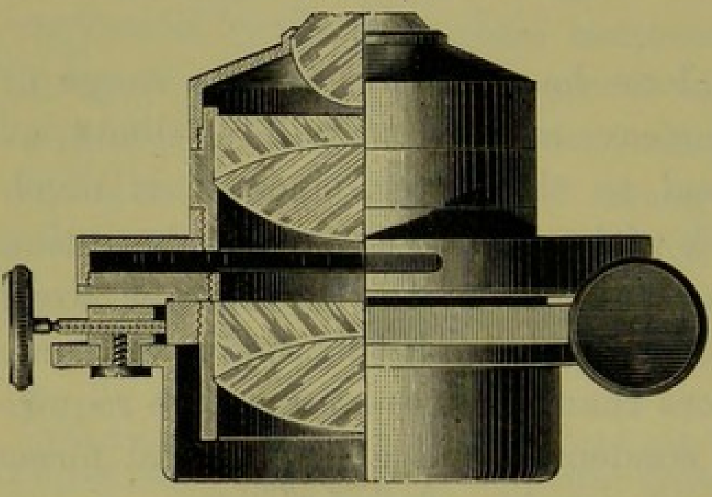


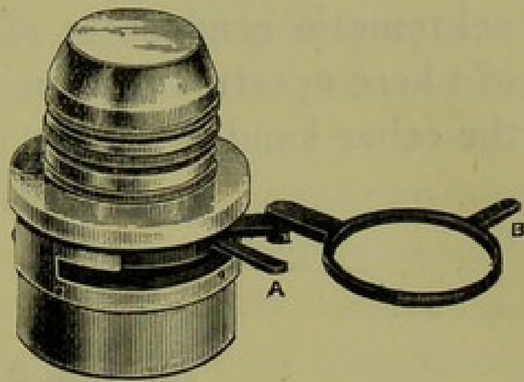
Fig. 17.



any one of them would satisfy the needs of the average worker. The genuine article costs about £3.

A condenser as free from spherical and chromatic aberration as an objective of good quality is still a desideratum. The nearest approach to it is probably to be found in using as a condenser an objective of somewhat lower power than the one attached to the microscope tube. Very fine results have, for example, been attained by using the 16 mm. apochromatic as the condenser for the 6 mm., where the loss of aperture was not a great consideration, and better results still would probably be got by using the 6 mm. as the condenser for the 4 mm., or the 3 mm. for the 2 mm., since in these two cases respectively the aperture of the pair of lenses is the same. The writer has also used two 2 mm. lenses, one below the stage, the other upon the tube, and the result was highly satisfactory.*

FIG. 18.




There must always be a considerable sacrifice in aperture where a dry condenser is used to illuminate an immersion lens, and indeed some objects refuse to reveal their structure under any illumination obtainable from a dry condenser.

The writer has occasionally used the Abbe-Zeiss achromatic condenser as an immersion condenser by "building-up" to the required extent (since the immersion working distance is far longer than the dry

* The object was in this case mounted upon thin glass, and not upon an ordinary slip. Both condenser and objective were immersed in oil.

one) with alternate layers of No. 3 thin glass and cedar oil. The condenser worked well under these conditions.

No dry condenser can have an aperture greater than N.A. 1, and it may be remarked that Zeiss's non-achromatic condensers of N.A. 1.25, and 1.4 are only of those apertures when "oiled-on" to the slip. On the other hand, when an object is mounted dry upon

FIG. 19.

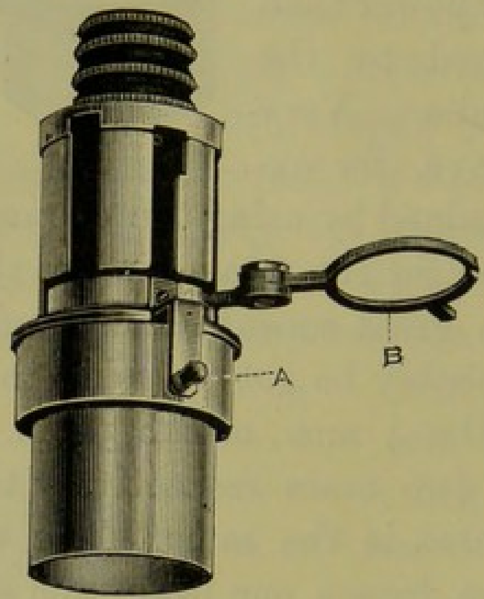
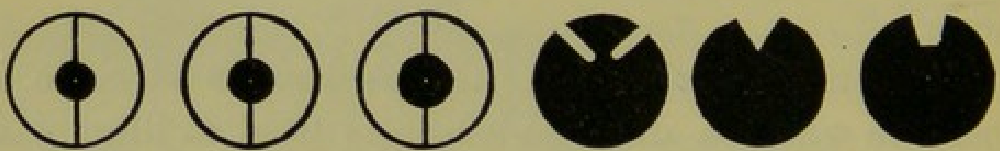



FIG. 20.



the cover-glass, it is of no service to use an immersion condenser, since the oblique rays never enter the layer of air beneath the cover-glass, but are totally reflected at the upper surface of the glass slip.

The finest immersion condensers (and the most expensive) are those, achromatic and apochromatic respectively, manufactured by Powell & Lealand, the former of which, with its stops, is figured here (Figs. 19 and 20.) The latter, almost identical in appearance, represents the highest development of the optical art as applied to this special purpose, and by its aid the very finest diatom workhitherto done has been produced.

A word or two may be added here on two minor pieces of apparatus, the micrometer and the finder.

The eyepiece micrometer is of course quite unsuitable for photo-micrographic work, since the value of its divisions varies with each several objective and with every variation in tube-length. It would be very convenient to be able to photograph the object and the micrometer scale at the same time, but whilst an eye-piece micrometer which would allow of this is conceivable from a mechanical standpoint it has not yet been constructed, and must needs be very complicated. We therefore have recourse to the stage micrometer, which should be divided into hundredths and thousandths of an inch, and tenths and hundreds of a millimetre. One point as to its use requires to be noted. The rulings themselves are usually made up of two, often of three, parallel lines, so that the division must not be measured by the distance between the last line of one ruling and the first of the next, but taken from a given line of one to the corresponding one of the next. In the case of the divisions of one hundredth of a millimetre the adoption of the inter-space as the distance would involve an error of 30 per cent in a single division. The exact method of using the micrometer in photo-micrographic work will be described in a later chapter.

In looking over a set of slides, or a single slide, it will often happen that particular fields are found which it is desired to reproduce subsequently by photography. For this purpose there is practically only one instrument available, the well-known "Maltwood" finder, which consists of a square of glass on which are photographed 2500 squares, numbered successively, in both vertical and transverse directions, from 1 to 50. Thus the first vertical row will read 1–1, 1–2, &c., the first transverse one, 1–1, 2–1, &c.; and so on.

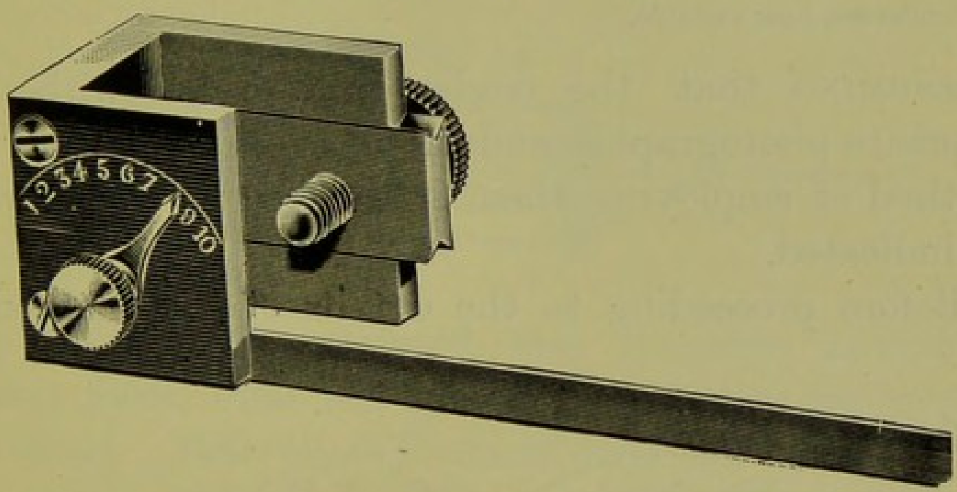
In microscopes furnished with a mechanical stage it is only necessary to place the slide against the stop to begin with, and then, when a desirable field is found, to substitute the Maltwood finder for the slide (taking care that it, too, is in contact with the stop), to read off the number which is in the field, and write it upon the label of the slide itself, with an arrow to indicate which end of the slide was against the stop. By reversing the process, placing the finder on the stage, with the number required in the field, and then substituting the slide for it, the field desired can be easily re-found.

In microscopes with plain stages, this procedure is impossible, and the enormous waste of time involved in hunting for a single field in a slide containing from 500 to 1500 fields, induced the writer to contrive the apparatus shown here (Fig. 21), which renders the Maltwood finder available on any microscope with a plain stage. When the desired field is seen, the slide is firmly fixed by the clips, the long thin bar of the apparatus is then inserted beneath the clips, and the rectangular elbow formed by the junction of this bar with the body is closely applied to the slide.

This being made sure of, the screw beneath is tightened, so that the frame is firmly held in position. The slide is now removed, the finder substituted for it, and the number read off and marked on the label with the same precautions as before. When it is desired to re-find the field, the apparatus is placed upon the stage with the finder in the angle, the required number found, the frame fixed in position, and the slide substituted for the finder. The desired object will then occupy the centre of the field.

The nut of the tightening screw slides in **V**-shaped grooves to allow of the employment of the apparatus for preparations with large cover-glasses. The index is intended to be placed vertically to mark the position of the slide upon the stage, but has not been found necessary in practice. The writer entrusted the embodiment of his ideas to Mr. C. L. Curties, under

FIG. 21.




whose supervision they were most satisfactorily worked out, and the apparatus may be obtained from Baker, but any good optician could produce it from the figure here given. The cost of the apparatus complete with finder, would be about thirty shillings. The finder itself is made by R. & J. Beck. It is well to practise the use of it with low powers; when very high magnifications are being employed, a lower power should be substituted in reading off the squares, care being taken that the object required to be re-found occupies the centre of the field.

## CHAPTER VII.

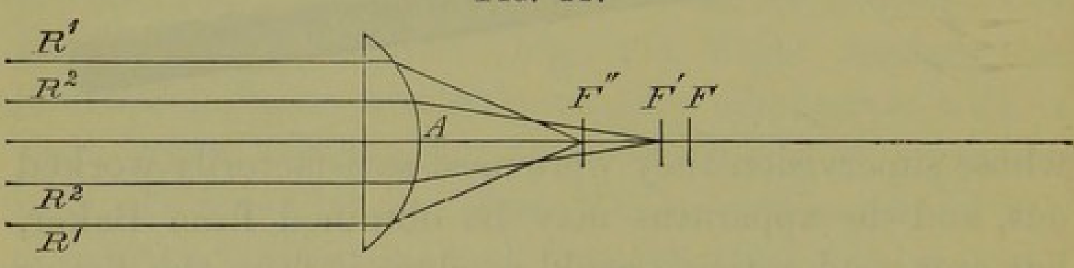
### ILLUMINATION.

General remarks on lenses and their action on rays of light—Spherical and chromatic aberration—Principal focus—Conjugate foci—Radiants—Sunlight — Monochromatic light — Heliostat — Lime-light —Flash-light—Lamp-light—Production of critical image—Centring of condenser—Controlling aperture of condenser—Use of bull's-eye—Fog—Focus of condenser, how variable.

PRESUMING that the reader is provided with the requisite photographic and microscopic appliances, the method of employing them in conjunction has now to be indicated.

Before proceeding to the details of the method, a

FIG. 22.

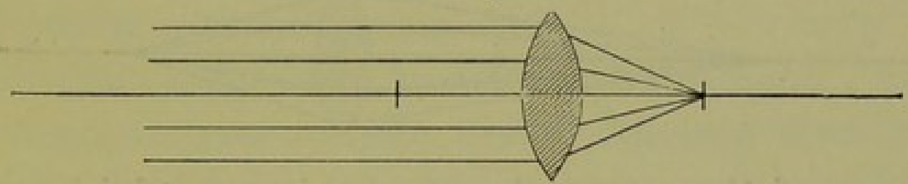



few elementary remarks upon the optical principles involved may be of service to the general reader.

Every lens has of course a focus, and this focus is theoretically a point, but in practice it is found that a simple lens whose surface is part of a sphere, is subject to two forms of "aberration," the first being the spherical error (shown in Fig. 22), due to the fact that the lens acts upon rays of light simply as a concentric series of prisms, which deflect the rays to an extent increasing from the centre outwards, the result being, as shown in the diagram, that the rays which pass through the margin are brought to a focus considerably nearer the surface of the lens than those which pass through the central portion. The thinner the lens, and the weaker its curvature, the less is the spherical error.

The second aberration is the chromatic, which arises from the fact that the different rays which form the spectrum are separated by the prisms of which the lens is made up, and that the rays so separated are not brought to a focus at the same point, the violet

FIG. 23.



rays being more refracted, and therefore having a shorter focus, than the blue, the blue than the green, and so on. Both these forms of error are well seen in an ordinary bull's-eye, and better still in a chromatic Abbe condenser.

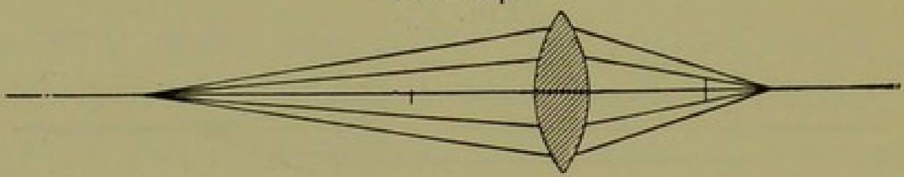
Every convex lens has, moreover, three foci, a "principal" focus, to which it converges parallel rays (Fig. 23); and two "conjugate" foci (Fig. 24).

For our present purpose it is less important to consider the power of the lens to converge parallel rays to its principal focus, than to note the converse position, that when a radiant is placed at the principal focus of a convex lens, its rays are parallelised in passing through the lens, no image being formed, but all the rays being converted into a cylindrical bundle. We shall have frequent reason to refer to this property hereafter.

The conjugate foci, as their name implies, stand in a direct relation to each other. When a convex lens is set up with a radiant on one side of it, and a screen on the other *at the same distance*, the distance being that at which the image of the radiant is sharply defined upon the screen, the relation of the conjugate foci is the simplest possible, since the radiant and screen are each at a distance from the optical centre of the lens equal to twice the focal length of the latter, so that half this distance gives the "principal" focus of the lens.

As the radiant is moved nearer to the lens, the

FIG. 24.



distance of the screen has to be increased in a much more rapid ratio to form an image which is still sharp, and on the other hand, if the radiant be removed from the lens, the screen has to be approximated to it, but far more slowly than the radiant is withdrawn.

Wherever under these conditions we have a radiant on one side of a lens and a sharp image of it upon the other, the two occupy conjugate foci, the position of which, with reference to each other, may evidently be varied to any points between parallelism and the principal focus on the one side or the other; and as regards the conjugate character of the two, it is a matter of indifference upon which side of the lens either may be placed. It will be seen, from what has been said above, that the lower the angle of the cone of rays which enters the lens, the higher is that which leaves it, so that if we place the radiant close to the condenser, we get a longer focus, and the cone thrown into the objective is of lower angle, whilst by removing the radiant to a distance, or employing parallel rays, we get the shortest focus and the highest angle of the substage condenser which are compatible with work under correct optical conditions. When the radiant is *within* the principal focus of the lens, we get not only a divergent beam, but total reflections inside the lens (Fig. 31, D), which render the cone no longer available for accurate work. The real conjugate foci have then ceased to exist. The foregoing principles all concern the elementary laws of optics, but if known to, they appear to be little applied by the average photo-micrographer, so that their introduction here may not be without its use.

The next thing to be considered is the light. Under this head we have to refer to four different illuminants, sunlight, lime-light, magnesium-light, and lamp-light.

For the employment of direct sunlight a heliostat is of course indispensable, and even with such an instrument, our English climate is so uncertain that the photo-micrographer who relies upon it will for a great part of the year be practically debarred from working. There can, on the other hand, be little doubt that it is not only the quickest, but on the whole the best in results. Its richness in the actinic rays is of very great advantage, since these rays not only impress the plate more quickly, but also, from their intensity, allow of the employment of monochromatic light by the interposition of a thick layer of ammonio-sulphate of copper solution or other light-filter. When monochromatic light is used, there is no longer any question as to the coincidence of the visual and actinic foci, any objective which forms a sharp visual image will also yield a sharp photographic one. If the light-filter selected be a solution, it should be used in a deep flat-sided cell, and not in a globe as is so often recommended. The globe, of course, acts upon the parallel rays of the sunbeam as a condensing lens, and the rays are thereby rendered strongly convergent before they enter the substage condenser, the focus of the latter being thus greatly shortened, very possibly to such an extent as to prevent it from coming to a proper focus at all.

Another very excellent means of obtaining light which is practically monochromatic is the interposition of a copper-chromium filter, made by taking 160 grammes of pure dry nitrate of copper, and 14 grammes of pure chromic acid, and adding to them water sufficient to make a total volume of 250 cc. Dr. Neuhauss remarks that in a layer half an inch thick this filter allows only greenish yellow rays of wave length 570–550 to penetrate it, so that its use is, of course, confined to the production of an image on orthochromatic plates, preferably those bathed with erythrosin solution. It is, however, the most truly monochromatic of all the modifications of light produced by coloured filters. A near approach to it may be obtained by the employment of orange and "signal-green" glass together. There remains still another method of obtaining monochromatic light—viz., the use of a prism (Fig. 25), and this is theoretically in every way the most perfect. The prism should be of quartz, if possible, since that material produces relatively a much longer spectrum at the violet end than glass or bisulphide of carbon, and whilst it is true, as above stated, that with monochromatic light the visual and the projected images are equally sharp, it is also the fact that the defining, or rather, resolving power of the

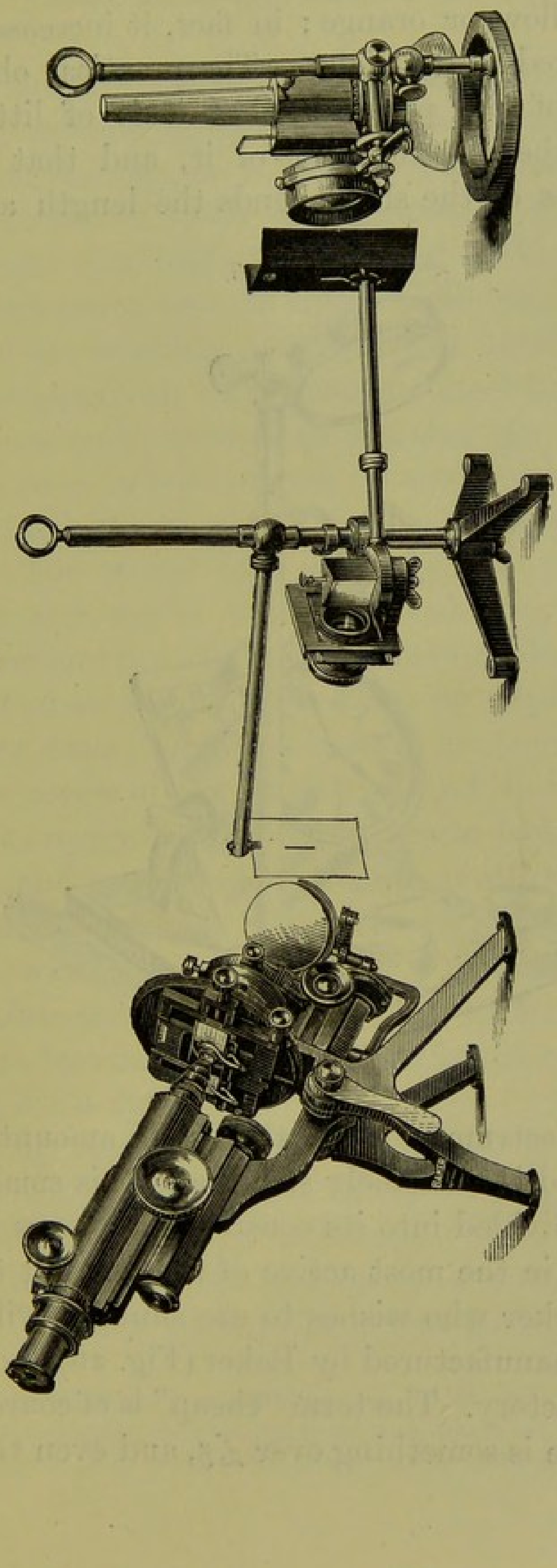
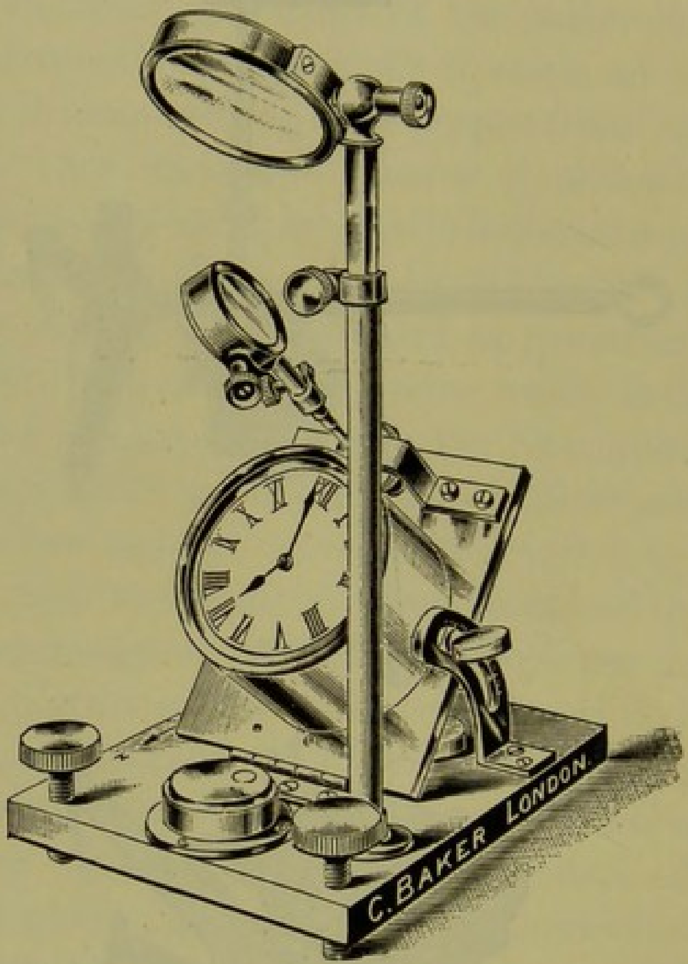


Fig. 25.

violet and blue rays is considerably greater than that of the yellow or orange; in fact, it increases steadily from the red to the violet. The practical objection to the use of the prism is that it is of little service without the slit in front of it, and that upon the narrowness of the slit depends the length and purity

Fig. 26.




of the spectrum. Thus the total amount of light transmitted is extremely small, and this small amount being subdivided into its constituent colours the exposure, even in the most active of them, is apt to be long.

The worker who wishes to use sunlight will find the heliostat manufactured by Baker (Fig. 26) at once cheap and satisfactory. The term "cheap" is of course relative —the price is something over £5, and even this is very much lower than that of any similar instrument in the market.

Next to sunlight comes without doubt the oxy-hydrogen, oxy-calcium, or lime light. This is the writer's sheet anchor, and oxygen is now so cheap that the cost becomes a matter of small consideration. Ten feet of Brin's oxygen cost half a crown, and as the average rate of consumption may be taken to be less than half a cubic foot per working hour, ten feet should take a good many negatives. The cost of a steel bottle with proper valves and connections is under £2, and this amount is soon saved in hire where much work is done, and still sooner where the gas is only used at intervals. The writer uses a "mixing" jet, and a hard lime, and works with the ordinary house-gas from a convenient tap above the camera-table. He has never yet had an accident, nor does he expect to, but at the same time there is a certain amount of risk. Should the nozzle of the jet get blocked, it is conceivable that the oxygen might obtain access to the domestic supply, and an explosion of some, or all, of the pipes would naturally follow. Such an event has been known to happen with a mixing jet, and it is therefore safer to use a "blow-through" jet and a soft lime. The light is somewhat less intense and less perfect in colour, but the safety is absolute.

Even with the mixing jet the danger is minimised if a regulator, costing about thirty shillings, be employed. This is really a reducing valve, interposed between the valve on the gas-bottle and the delivery tube, and its use is much to be recommended.*

* A hint on the preservation of the limes may be of use. When a tin is opened, the whole number, save the one to be used, should be put into a warm, dry, wide-mouthed glass bottle, with a well fitting cork, and the cork be

Each exposure "pits" the lime, and this affects the intensity of the light, so that the lime must be slightly turned for each exposure, in order to obtain a light of constant intensity, a matter of considerable importance when an over or under exposure has to be corrected by a fresh one.

The mode of procedure is as follows: the lime being placed upon its rod, the hydrogen is lighted and the lime placed almost in contact with the jet. The hydrogen is turned up or down, so that a flame about an inch and a half in height plays on the lime, and the oxygen being now cautiously turned on, a spot of intense white light is produced. The hydrogen flame sinks down almost to nothing, but the proportions may be judged from the width of *red* flame visible at the top of the flame of the mixed gases. If the red band be not visible, the oxygen must be turned down until it just appears, or a sharp explosion or series of explosions will be followed by the apparent extinction of the gas, which is really burning inside the mixing-box of the jet. The remedy is to turn off the oxygen and start again as at first.*

Magnesium has almost entirely lost its place as an illuminant for photo-micrography, though it is possible that the flash-light may be of value for low-power

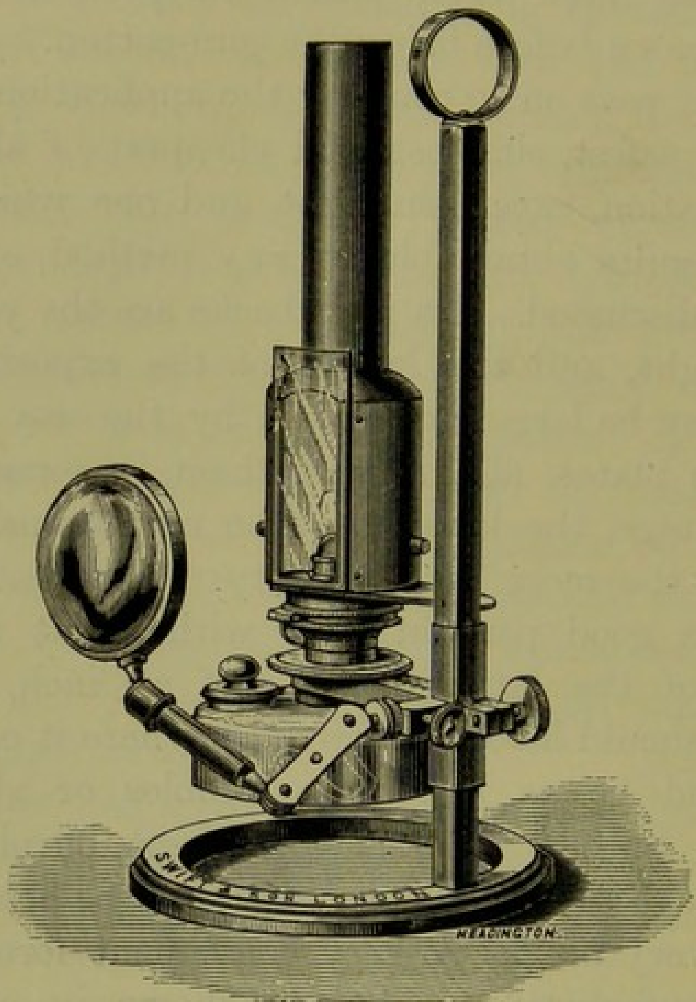
dipped two or three times into melted hard paraffin, or be thoroughly sealing-waxed, after closing the bottle. The least pin-hole will allow of the entrance of moisture and the limes will then slake and fall to pieces. The powdered lime contained in the tin is next to be put into a wide-mouthed glass-stoppered bottle, also dry and warm, forming a bed upon which, during the intervals of work, the cylinder in use reposes. The writer always lays his lime upon this bed while it is hot, and the consequence is that a single lime will serve him for a hundred exposures.

* Mr. Pringle's jet offers very great advantages as regards the obtaining of the same amount of light with successive exposures. It may be purchased from Newton, Temple Bar, or from Baker.

work with moving objects. In this case the proper method is to place a little loose gun-cotton on a stand in the optic axis, after everything else has been got ready, and the object placed in position. Upon the gun-cotton a sufficient quantity of powdered magnesium is placed, the two mixed, and a match applied. It may not be amiss to remind the reader that the plate must have been placed in position and the shutter drawn before firing the gun-cotton.

We now pass on to consider the application of lamp-light, the safest, simplest, and cheapest of all sources of illumination, except sunlight, and one which yields all the results obtainable by any method, except the one last discussed. Its drawbacks are the yellowness of the light, and the length of the exposure. The former may be largely overcome by the use of orthochromatic plates, and is, with them, in some respects an advantage, the latter may be met by using these plates in the more rapid quality. The best form of lamp is a good paraffin lamp with a flat wick, the breadth of the latter being half an inch, and the chimney should have parallel sides where it covers the flame, and be as free from bubbles or streaks as possible. Such a lamp may be kept burning for a week at a cost of sixpence, and can be made to do almost everything. A very convenient form of it is that manufactured by Baker (shown in connection with the writer's apparatus in Fig. 5, where it is also provided with an achromatic bull's-eye), in which the lamp itself slides upon a rod, and the chimney is of metal, with grooves for the reception of an ordinary thin glass microscopic slide, or of slips of coloured glass of the same size. Every microscope maker, however, will supply one suitable for the purpose, the price being regulated by the complexity of the structure and the character of the workmanship. Here again it may be noted that any lamp of suitable height will answer the purpose; and whilst it is an undoubted advantage to have a dark chimney, this may be satisfactorily constructed of asbestos paper, or of tin (the

Fig. 27.



writer's is of asbestos paper), of a size somewhat larger than the glass one. It is well to have a cover arranged about two inches above the top of the chimney, to stop reflection from the ceiling. The less extraneous light there is in the room the better will the microscropic image be seen, and the more sensitive will the eye be. A hole is of course to be cut in one side of the chimney to correspond with the position of the flame, and should be at least two inches in diameter, to allow of the use of the bull's-eye.

In working with very low powers it is advisable to use a larger lamp, and for this purpose the writer recommends Dietz's $1\frac{1}{2}$ inch Paragon burner.* He has had his own attached to a tin reservoir holding about three pints of oil, and of such a height that the centre of the flame, when $1\frac{1}{2}$ inches high, corresponds with the optic axis of the microscope when the latter is horizontal.

Whilst there is, perhaps chiefly owing to the influence and reputation of Mr. E. M. Nelson, a strong feeling in this country in favour of a flat flame for photo-micrographic work, some of the very finest continental work is done with round flames, and it is such a lamp which Dr. Neuhauss uses for the production of his remarkable results. For this reason the writer does not feel inclined to dogmatise upon this subject, but he does not hesitate to express a strong predilection in favour of the use of the flat flame on edge for all work but that done with powers of a focal length exceeding 2 inches.

Having dealt thus at length with the means of illumination, the method of employing them to the best advantage has next to be considered, and here, as in the first edition of this book, the writer hastens to express his indebtedness to his friend Mr. E. M. Nelson, whose papers upon this subject,† coupled with oral information ungrudgingly given, directed the writer into the proper road at the outset of his work, and have had much to do with any measure of success which he has attained.

* It may be obtained from R. & J. Slack, Strand.

† See especially Jour. Roy. Micr. Soc., Aug. 1885.

The method will perhaps be best understood by detailing the steps of the process as applied to a well defined object, for instance, a round diatom of considerable size and strong markings, such as *Aulacodiscus Margaritaceus.*

Applying to the microscope the condenser, a one-inch objective and a medium eyepiece, the lamp is placed in front of the microscope at a distance of about a foot, and its light reflected by the plane mirror in the axis of the microscope (Fig. 28). Or the condenser may be

FIG. 28.

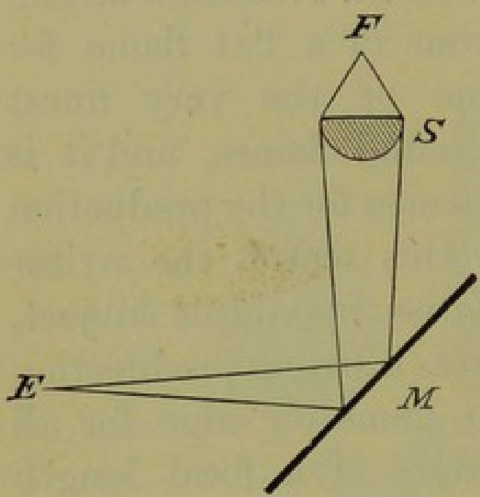
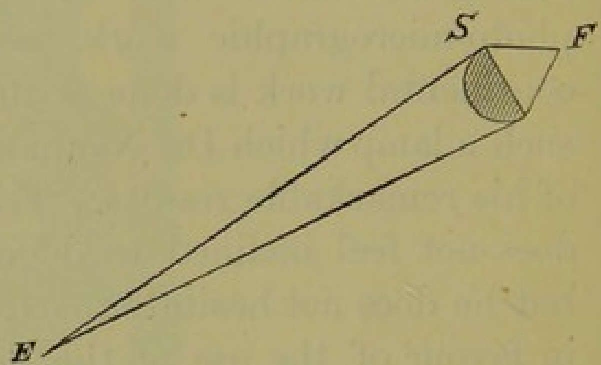
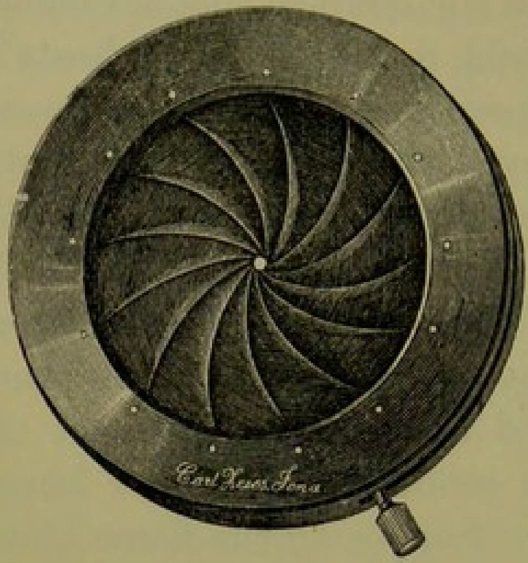



FIG. 29.




pointed directly toward the edge of the flame of the lamp (Fig. 29), which is the better, though with most stands the less convenient plan. It has the advantage of doing away with false images produced by the reflection from the front of the mirror. The object being now placed upon the stage and focussed, the condenser is to be moved up or down until a sharp image of the flame is projected across the vertical azimuth of the diatom. If the image of the flame be sharp, but not vertical, the mirror is not central; if the image be vertical, but on one side of the centre of the field, either the lamp or the condenser is, or both are, out of centre. To remedy the position of the lamp is an easy matter, but the centring of the whole system is accomplished by the writer as follows. He begins by employing an objective weak enough to take in the whole area of the front lens of the condenser, then focusses down upon the latter and centres it, and arranges the lamp so that its flame occupies the centre of the field, and that no "ghosts" of the flame, due to false positions of the mirror, are visible. As the mirror is moved, these ghosts, if present, will move too, and when all is central they will correspond with the image of the flame and be lost sight of. When a higher objective is substituted for the low one, the flame may be somewhat out of the centre, and this is corrected by slight adjustment of the centring screws of the condenser. Neither lamp nor mirror must be shifted. Supposing the flame thus properly centred, and in focus upon the object, bisecting the image of the latter, it is extremely probable that the image of the diatom will appear to be drowned in light where the image of the flame crosses it. This will be due to one of two causes, excess of light beyond what the eye can bear, or a cone of rays exceeding the aperture of the objective, or at all events the available aperture. If it be due to the first of these two causes, it will be removed by the simple interposition of a blue glass screen, of the tint known as "optical blue." If, on the other hand, the want of clearness be due to excessive aperture in the condenser, there is but one remedy—viz., the insertion of a stop, if stops be used, or the closure of the iris diaphragm (Fig. 30) to the required extent. When the flame and the object are both sharp the relation of the condenser to the objective may be easily ascertained by taking out the eyepiece. On looking down the tube the back lens of the objective will be seen as a disc of light. If this disc extend to the very edge of the lens, the objective is filled, or overfilled—that is to say, the aperture of the condenser is equal to, or greater than, that of the objective. If the aperture of the condenser be less than that of the objective, the disc of light will be surrounded by a dark ring representing the portion of the aperture which is not being utilised. There are comparatively few lenses, save among the apochromatics (which in this respect form a distinguished exception) which will bear *filling* with light—that is to say, of which the whole of the aperture is available. The exact amount to which any given objective requires to be stopped down can only be determined by trial, and once known there is no trouble in the matter, beyond the loss of light and of defining power inseparable from lessening of aperture.

FIG. 30.

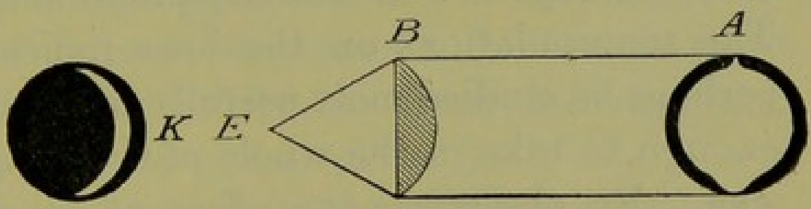



Supposing then the light, condenser, and object to be centred upon the optic axis, the image of the flame to be sharply in focus with that of the object, and the condenser and objective to be giving and receiving respectively a cone of rays of the width most suitable to the objective in use, there yet remains the question how the band-like image of the flame is to be made to cover the whole or a sufficient part of the field with an even illumination. The image of the flame illuminates so small a portion of the field of a low power that a photograph so taken would be a very inartistic production.

The bull's-eye here comes to the rescue, and enables us to overcome the difficulty completely. Without disturbing any part of the arrangements already made, let the bull's-eye be interposed between the lamp flame and the condenser (or mirror, if this last is being used). The centre of the bull's-eye must be upon the line joining the centre of the flame and the centre of the mirror (or of the condenser), and its plane surface at right angles to this line and toward the flame.

Fig. 31.


Fig. 32.




Placing the bull's-eye as far as possible from the lamp upon this line the image of the flame will still appear, but drawn out into a straight or spindle-shaped band of light (Fig. 31, H), and, as the bull's-eye is brought nearer to the flame, this band will gradually widen into a disc which will evenly illuminate the whole field. Variations to one side or other of the central line will be indicated by corresponding deformities in the shape of the band (Fig. 31, K), whilst when the bull's-eye is too near the lamp the disc is no longer even (Fig. 31, D); it should be pointed equally above and below, and curved equally on both sides. When the even disc (Fig. 32, A) has been obtained, it is necessary to move the condenser somewhat nearer than before to the object, since the bull's-eye is now sending to it a bundle of parallel rays, which it will bring to a focus at a point nearer its own front surface, and therefore farther from the object than was the case with the divergent rays which it previously received. There is now no image of the radiant to guide the observer, and he must therefore watch carefully the amount of light in the field. The condenser will be in focus when this light reaches its maximum ; above or below the focal point the light diminishes rapidly.

The worker is strongly recommended to spare no pains to familiarise himself with the use of the bull's-eye, which is, when properly employed, one of the most valuable adjuncts in ordinary photo-micrographic work. The manipulations on the lines indicated above may perhaps be studied most usefully with an objective weak enough to take in the whole of the image of the bull's-eye. By observing the effect of placing the lens in various positions between the lamp and the condenser the proper method and position will soon be arrived at.

With sunlight and the heliostat, the rays being already parallel, very little good can be expected from the use of the bull's-eye. With the flash-light, again, its use is contra-indicated. All rays of light travel through lenses in a well-known manner, but he would be indeed a skilful operator who could so arrange his gun-cotton and magnesium as to produce a disc of light like that yielded by the bull's-eye when the position of the radiant is a fixed and definite one. With lime-light and lamp-light, on the other hand, the results described above can always be obtained. Let it be distinctly understood that what is called "fogging," a *woolly* appearance of the image in the microscope, easily recognised after being once seen, is never due to excess of light, as is easily proved in the way previously mentioned, by using a blue glass screen sufficiently dark to render the light bearable to the eye. Woolliness, or fog, can never be removed by this means. So far as the writer's experience goes, it is always due to one of two causes, both of which are to be sought for in the objective alone. The first is, inability to bear the aperture of the condenser. This is to be remedied by stopping down the latter as much as is necessary. The second is dirt, and this may be either on the front lens of the objective, or somewhere else in the combinations of which the objective is made up, and the remedy is its removal. The writer does not include under the term "fog" the general haze which in dry or water-immersion objectives of high aperture results from inadequate correction of the objective for thickness of cover-glass. This is dealt with in its proper place.

In concluding our account of the bull's-eye, we must again refer to the fact that it may be successfully employed as a substitute for the substage condenser for work with low powers. The bull's-eye being arranged beneath the stage in the optic axis of the microscope, the lamp is placed in front of it, with the edge of the flame toward it, and the relative distances of lamp and bull's-eye from each other and from the stage are varied until an image of the flame is thrown upon the object, which will then be brilliantly illuminated by a cone of rays of about 30° angular extent, sufficient for any objective of less focal length than $\frac{2}{3}$ inch. In fact, the bull's-eye plays a most important part in photo-micrography, and will well repay any amount of trouble spent in mastering the use of it. The flat side should always be towards the radiant.

When the power employed is so high that the image of the radiant thrown by the condenser into the plane of the object will cover the whole extent of the negative, or when the portion of the object to be reproduced is so small as to produce a similar result, the use of the bull's-eye is unnecessary, and in most cases disadvantageous. Though the total amount of light obtained by it is greater, the intensity on any given area is less than when the image of the radiant itself is brought into focus. In certain special circumstances, where the very utmost extent of the aperture of wide-angled lenses is to be utilised, the bull's-eye may be used to parallelise the rays from the radiant, in which condition the condenser brings them to focus at a still shorter distance from its front than when they are divergent on entering it.

It will occasionally happen that a slide is too thick to allow the image of the radiant to be brought to a focus upon the object. In this case the condenser must be brought up into contact with the under surface of the slide, and the lamp moved nearer to the mirror or the condenser, as the case may be. The length of focus of the latter may thus be increased to such a degree that the image of the radiant is formed in the plane of the object, and so the appropriate conditions of illumination are satisfied.

Finally, it may be noted as to the choice of a radiant, that the absolute certainty with which exposures may be corrected when lamp-light is employed goes far to compensate for the increase of exposure involved by its use. With medium orthochromatic plates, and a pale yellow screen, the writer finds seventy-five seconds sufficient at a thousand diameters.

# CHAPTER VIII.

## MICROSCOPE AND CAMERA IN COMBINATION.

Preliminary precautions—Magnification—Projection-ocular—Position of image—Focussing—Lenses for the purpose—The writer's form—Steinheil—Precautions.

THE details given in previous chapters having been mastered, the combination of the microscope and camera has now to be accomplished.

In describing the steps of this operation, the horizontal position of the whole will be taken for granted, the modifications necessary for the vertical one having already been referred to.

The microscope is first of all to be placed in position, and the extended-focussing mechanism connected to the fine adjustment. The condenser having been already centred when observing, the next step is to place the radiant so that its image occupies the centre of the field of view, and is in focus in the same plane as the image of the object, remembering that a thick slip may require the light to be very near the substage condenser. If the bull's-eye is to be used it must now be interposed, with its flat side toward the radiant, and adjusted as previously described, according to whether it is required to function as a substage condenser or simply to furnish a large and even bundle of parallel rays for the latter to concentrate upon the object. The lighting must be made as even as possible, and the method which the writer adopts is to hold a white card about six inches behind the eyepiece, focus the object upon it, and spare no pains to make the whole disc of light of the same intensity, or at any rate to ensure this for the part of the field which is to be reproduced. Many a negative is ruined by uneven lighting alone, and the sensitive plate will record and repeat differences which are absolutely unnoticeable by direct observation. The ground-glass screen furnishes a most untrustworthy criterion upon this point, and the writer never thinks of relying upon it, but invariably proceeds upon the lines above described.

If the magnification have not been previously measured or decided upon, it should be attended to before connecting the camera to the microscope, in order that the correction-collar of the projection-ocular may be accessible, supposing that ocular to be in use.

If the object is to be photographed at a given magnification, the length of camera must have been previously determined. Indeed, the worker will find it a very great convenience to make a table of the lengths required with various objectives and eyepieces in combination. No two objectives are exactly alike in initial magnification, and for scientific measurement it is necessary that the magnification be accurately known.

If a projection-ocular be used, again, the correction required varies widely, and this affects the result. For instance, the writer finds that to obtain a magnification of 1000 diameters with 2 mm. apochromatic objective and No. 3 projection-ocular, he requires a distance of 25.5 inches from the eyepiece point (*i.e.* the flange of the eyepiece where it abuts upon the tube of the microscope) to a fixed point upon the camera. The index of the projection-ocular at this distance stands midway between 5 and 6 upon the scale, and any variation in the distance has two results. The one is that the image of the diaphragm is no longer sharp upon the screen, the other that the magnification is altered. In order to arrive at the result above given, the writer used an ordinary stage micrometer, divided to .001 of an inch, and varied the length of the camera and the collar of the eyepiece slightly until the image of the diaphragm was sharply projected on the screen when one division of the micrometer corresponded exactly with one inch upon an ordinary rule.*

The practice of photographing at a known magnification is strongly to be recommended, and adds largely to the scientific value of results. Should the absolute magnification be of less importance than the size of the picture, the magnification may be readily obtained afterwards by leaving the camera alone, and putting a micrometer in place of the object. If, then, its image be focussed upon the screen the magnification can be readily calculated. Thus, if the projected image of a division of .001 of an inch measures three-quarters of an inch, the object, under the same conditions, was magnified 750 diameters. This, whether carried out before or after exposure, is the most absolutely certain method known to the writer, and appears to him far preferable to measurements by eyepiece micrometers or by calculation from focal and tube lengths.

The light being now arranged, the magnification known, and the camera coupled up to the microscope,

* Since the adjustment of the projection-ocular depends entirely upon the distance between it and the screen, it will save much inconvenience to find out once for all the distances which correspond to the divisions of the ocular in question.

the next thing is to see that the image is in proper position. This the writer invariably effects by putting in the dark slide, opening it, and inserting a piece of ground glass, exactly the size of the plate which is to be used, behind the mask previously recommended. The position of the object on the negative is thus absolutely known, and much time and subsequent disappointment are saved.

If the position be satisfactory the next stage is to focus. If it be not, the camera (or, in the writer's apparatus, the adjustable back, see p. 23) is to be shifted until things are put straight, always keeping at right angles to the optic axis. The worker must remember that his light has been adjusted to the centre of his object, and the latter must not be shifted,* since it is beyond everything desirable that the centre of the field of view should coincide exactly with the centre of the plate. It must be remembered, too, that light, bull's-eye, condenser, object, objective and eyepiece, have all been arranged as nearly as possible on the optic axis, and if one of them be disturbed the whole arrangement is vitiated.

It now remains to focus the object sharply upon the ground-glass screen, so far as the eye can judge, and then, removing the ground glass and dark slide, to insert the clear-glass ruled screen already referred to.

We are now at the critical point of the whole process, and that being so, we call in fresh resources to meet the exigencies of the case. Upon the clear glass no image is of course visible; and we therefore have recourse to a focussing glass, by which the rays forming

* Except as may be required in consequence of the desired portion not occupying exactly the centre of the field, as explained in treating of curvature of the cover-glass, pp. 23-24.

the aërial image which we have to use to impress the plate are brought to a focus upon our own retina. All sorts and conditions of focussing glasses have been proposed, but one thing is certain—the less the form chosen magnifies, that is to say, the more nearly it shows the image as it will strike the plate, the better will the result be.

The form used by the writer is his own. Having tried successively the Ramsden, the Steinheil and the Huyghenian eyepiece, he has settled down definitely to an ordinary double-convex lens (a spectacle lens really) of about eight inches focus, mounted in a brass ring deep enough to keep it from being scratched when laid down, and costing complete half a crown.*

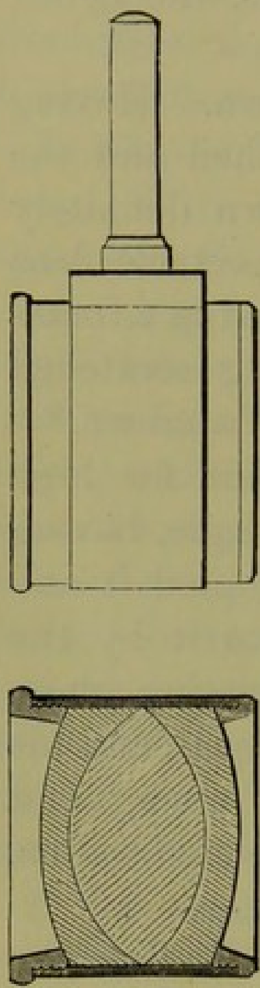
Its use is a little awkward at first, but for high power work it is invaluable, and Mr. Pringle, having seen it in the writer's hands, at once adopted it and uses it regularly. The writer was led to it by the fact that the lower the power of his focussing glass, the more readily were the fine details grasped, and his final conversion was accomplished by seeing and "holding" with it the flagellum of the cholera bacillus, which he could not see with any other.

The only difficulty in its use consists in finding the proper distance from the eye at which to hold it. In all other forms the eye is placed close to the lens; in the writer's, in consequence of the considerable focal length, the case is different. The easiest way is to hold the lens between the eye and the lamp and find the distance at which the whole ring is filled with light. Having learnt this, the rest is simple. The lens, at about the ascertained optimum distance from

* It may be obtained exactly in the form used by the writer from W. F. Stanley, London Bridge Approach.

the eye, is directed to the ruled screen, and so held that when the ring is filled with light the ruling is sharply in focus. The secret of focussing is to get the image at its maximum sharpness when the rulings are in the same condition, since the plate will occupy exactly the position of the latter.

Fig. 33.



With a long camera the image-forming rays are so nearly parallel that a comparatively large difference in position has no effect upon the sharpness of the image, though it has upon magnification. With a short camera, when the rays are more strongly divergent as they form the image, this is not the case, and it is very important to ensure that the focus of the image shall be in the plane of the rulings. With low magnifications, again, the Steinheil (Fig. 33) is more convenient than the writer's focussing glass, and he often uses the former, but as he does fifty negatives at a thousand diameters to every one under five hundred, the Steinheil is not often called upon, and after a little practice a convex lens of about four inches focus will be found superior to the Steinheil.

For tube-culture photography, to be referred to hereafter, the Steinheil is practically indispensable, and the writer always employs it. And he would add that he considers it preferable, after adjusting the position as above described, wherever the mask is used to cut off a portion of the field, to substitute a piece of ruled glass of the same size for the ground glass (or any scratches or even finger marks will serve as a guide upon which to focus the focussing glass), and to focus exactly the centre of the field to be photographed, a point of the greatest importance when the field of the objective (especially the case with apochromatics) is not flat, as otherwise the sharp part of the negative may be out of the centre.

The actual focussing is, of course, effected by working the coarse adjustment (with low powers), or the fine adjustment (with high ones), the latter by means of an extended-focussing arrangement when necessary. No amount of description will make the worker proficient in this matter, no amount of time and care is too great to expend upon this final microscopic act, if the best result is to be secured. The writer does not hesitate to spend half an hour in satisfying himself, with a difficult object, that he has the very sharpest focus of it attainable, and it is an absolute rule with him never to waste plates on images which are not as perfect as he can make them.

Nor are even the above precautions always sufficient. The insertion of the plate into the dark slide always should, and, if the directions given be carried out, always will, be left until the best focus has been attained. When the plate has been prepared for exposure the image is to be examined again to see whether the focus remains in the same condition as before. Alterations in the tension of the strings or bands of the focussing apparatus, or slight variation in the position of the spring of the fine adjustment, may happen in the interval, and the whole process may have to be repeated. The writer's tangential pulleys (p. 24), combined with very slow and gentle movement of the fine adjustment, will obviate this in the case of high powers. With low ones such minute precautions are not required; in fact, with magnifications below 400 diameters re-examination of the image, though advisable, is not necessary, and it is only with those above 750 that any alteration is likely to be called for.*

The dark slide is now to be inserted, and this must be done with the utmost care and gentleness, and the same remark applies to the raising of the slide which intervenes between the plate and the object. But before this is done, it remains to be determined how long the plate is to be exposed to the image-forming rays.

The secrets of producing a successful negative are, proper and even illumination, accurate focussing, and adequate exposure. We have endeavoured to explain how the first two conditions may be satisfied. The third, and the development of the impressed image, will constitute the subject of the next chapter.

* The writer has been reproached with not having, in the previous edition, furnished instructions how to focus. He thought he had ; but, at any rate, he has done his best, in the above directions, to meet every difficulty likely to be met with, or which he has himself had to overcome.

## CHAPTER IX.

### EXPOSURE.—DEVELOPMENT.

Its determination—The writer's table—Its employment—Warnerke's sensitometer—The critical moment—Removal of the plate—Marking it—Development — Over-exposure — Under-exposure — Density —Fixing—Washing—Clearing—Drying—Reduction—Intensification.

In the previous edition of this work, the writer embodied in the form of a table the result of numerous experiments which he had made to determine, as accurately as possible, the exact amount of exposure required with (1) a colourless object, (2) a plate of known sensibility, and (3) a light of known intensity. The table, in its original form, has been of service to many workers, but the writer has found it possible to simplify it very considerably, and in that form it is re-introduced here; an error of calculation which slightly affected the longer exposures has also been corrected.

The use of the table—in other words, the pre-determination of the exposure—depends upon two conditions: the sensitiveness of the plate, and the intensity of the light. Both of these are to be determined with one and the same instrument—viz., Warnerke's sensitometer screen, which may be obtained, at a cost of six shillings, from any photographic dealer, or from Baker. It is a plate of glass on which are numbered squares, each one darker than the one preceding it to an extent necessitating one-third more exposure to impress upon any plate the figure it contains than does the one which precedes it. In order to test the sensibility of a plate, a luminous tablet is required, which is excited by burning a few inches of magnesium ribbon about four inches in front of it. The sensitometer screen being exposed in a frame, with the plate to be tested in contact with it behind, the luminous tablet is placed close to the front of the frame, and is allowed to act upon the plate for exactly half a minute. The plate is then developed with a normal developer (p. 11), fixed and cleared in the usual manner, and the last number visible upon it when held up to diffuse daylight is the "sensitometer number" of that plate.* The obtaining of this sensitometer number is the first step toward the use of the table, and the latter is of little service without it.

The next step is to test the light to which the plate is to be exposed when an object is to be photographed. For this purpose, the size of the picture and the angle of condenser best suited to the subject having been decided upon, the object is to be moved just enough to leave the field clear. The sensitometer screen is to be held against the ground-glass screen, so that its numbered centre portion covers the centre of the field. A Steinheil, or other lens of the same sort, is held against the sensitometer screen, and the numbers are examined to determine the highest one which is legible by the light transmitted by the ground glass. We will suppose this to be 20, and that the sensitometer

* Plates of the same grade from one and the same maker will not be found to vary to an extent sufficient to require for each batch the repetition of this method.

number of the plate is 20 also. These two numbers added together give a total of 40, and in the table this (with other numbers obtained in a similar way by adding together the "sensitometer number" and the "last number visible") is given in the columns headed "No." Against each of these numbers is given, in seconds, or minutes, or hours, as the case may be, the exposure required to produce a certain effect. This effect is the printing of the number 14 of the sensitometer screen upon a plate placed behind the screen in the dark slide, and exposed (for the same time as the object would by the table be exposed) to the light visible upon the ground-glass screen with no object in the field of view. The results obtained will to some extent be affected by the sensitiveness of the retina of the individual worker's eye, but a little experience will show whether he requires to modify his exposures accordingly. An extremely sensitive eye may be able to read a higher number upon the screen than the average, and this will tend to under-exposure; with a less sensitive retina the result will be over-exposure. In the first case the number will be too high, in the second it will be too low, but supposing the eye used to have remained in darkness for two minutes before judging the light,* it will be easily determined how many places upon the table must be added or subtracted in order that the correct exposure shall be given. For instance, the exposure against 41 in the table is two minutes, but it may be necessary to give that exposure for 43 or 44, or it may be only necessary

* This is of considerable importance. An eye which has just been used for focussing an object by oxy-hydrogen light is certain to under-estimate the last number visible against the screen, probably to the extent of four or five places of the sensitometer.

to allow that exposure for 39 or 40. This will depend upon the sensitometer (which may vary slightly from the standard) or upon the worker's eye (which may be more or less sensitive than the average). Of course, after a long experience, it is not necessary to use the table regularly, especially when working at the same magnification, with the same light, nor will the table be an absolute guide in dealing with coloured sections, or when using coloured screens. In the latter case, however, the addition to be made to the "No." can be easily determined for any screen used, and will of course remain constant for that screen—that is to say, that if a given screen lengthens the exposure to the extent of three places on the table at any point it will do so at every point.* And now, supposing the length of exposure to have been determined, and given, the dark slide is to be gently removed, and in the case of high magnifications the focus again examined. If it have shifted, no good result need be expected; if it have remained steady, failure from that cause is out of the question.

The plate is now to be taken into the dark room. Before it is removed from the slide the developer should be mixed, taking for a normal pyro-developer one drachm of solution I., two drachms of solution II., two drachms of solution III., and three drachms of water. The slide is now opened, the plate reversed in it, and any required details written upon the margin with a lead pencil. It is both easier and better to do this before development, thus making every plate its own register. The silver salts in the film cause it to take the pencil far better than dry gelatine does after fixing.

* This remark applies to every correction of the table which the worker may find it necessary to make.

The plate is now to be laid, with the film side uppermost, in the developing dish. The dish being held in the left hand, tilted slightly away from the operator, the edge of the measure is rested upon the edge of the dish and swept along it from left to right as the developer is poured over the plate. This should be done rapidly, but gently, and air-bubbles will very rarely be a source of trouble. If any do adhere to the film they must be removed at once, preferably by sweeping them off with a soft camel-hair brush *kept for the purpose.* If not noticed and removed before the development has started there will be a clear spot in the negative for each one, and, of course, a dark spot upon the print.

The same remark applies to dust upon the plate, and for this reason the latter should always be well brushed with a broad camel-hair "softener" before insertion into and after removal from the dark slide.

If the proper exposure have been given to the plate, the image will begin to appear in from 15 to 30 seconds, the time varying slightly without appreciable effect upon the finished negative, but 30 seconds is almost the outside limit for a negative of fair printing density. Very much has been written to demonstrate the perfect ease with which an under-exposed negative may be brought up to full density, or an over-exposed one kept back, with maintenance of the desirable relation between the several component parts of the image, by suitably modifying the developer. It may be so in other departments of photography than those which have come under the notice of the writer; it is emphatically not the case with photo-micrographic negatives, and he is distinctly of opinion that it is, under all conceivable conditions, far better to correct a wrong exposure by means of one of proper duration than it is to attempt to "tinker" up a wrongly exposed negative. The final purpose of a negative is the production of a print, and perfect prints can only be got from properly exposed negatives. If the writer makes a wrong exposure, he carries the development out as if all were in order, examines the completed negative, learns all he can from it, not only as to exposure, but as to focus, position, and other details, and then makes another exposure, with a result generally more satisfactory.

If a negative shows no signs of coming up within $1\frac{1}{2}$ to 2 minutes, the proper treatment is to wash the plate at once and place it in the hyposulphite bath, making thereafter another negative with at least four times the exposure.

It may happen, in a subject presenting very strong contrasts, that certain portions flash up very rapidly; but even here it may be doubted whether the plate is not over-exposed, since in a negative taken by transmitted light the ground will usually be by far the brightest part, and should not appear within less than the time above stated. Supposing the image to commence to appear within the assigned limits, the development is to be carefully watched, the dish being constantly rocked to keep the fluid in motion until all detail is developed, and then one more drachm of solution III. being placed in the measure, the developer is poured into it, and the whole again swept over the plate. The object of this proceeding is to obtain the required density. This is altogether a matter of judgment and experience. It may be taken that as a rule no part of the image should be absolutely white when development is complete; still less should any part allow the light of the developing lamp to be distinctly seen through it. The test usually given—viz., that the development is complete when the prominent points of the image are visible at the back of the plate, is altogether fallacious, since it depends entirely upon the particular brand of plate in use, and plates which answer to this test extremely well will be found, generally, to give thin images when fixed. In short, the matter is emphatically one in which an ounce of practice is worth a pound of theory ; a little experience in the relations between negatives and prints will be of more value than pages of description. One point may, however, be noted, that the best negatives are those which require the addition already advised, one drachm of solution III. (making three drachms in all) to give them sufficient density. A negative which will not stand this addition is most likely over-exposed ; a negative which requires more is pretty sure to be thin when completed, in parts, if not all over.*

When the required density is reached the plate is washed in three or four changes of water, and placed in the hyposulphite solution. In this it should remain for at least five minutes after all white opacity has disappeared. It is then washed in running water for a couple of hours, if in a dish, or a quarter of an hour under a rose-tap, and placed for three minutes in the "clearing" solution. After any stain has been removed by this means, the plate is to be washed again for a somewhat shorter time, and finally dried in a rack, on edge.

On no account should a plate be stood upon a shelf

* Edwards' orthochromatic plates are best developed without the addition of one drachm of solution III. if possible. There is then much less liability to the production of green fog.

or table to dry. The surface tension of the fluid upon it carries up particles of dust and dirt sufficient to ruin the appearance of the very best negative.

One kind of stain is not removable by the clearing solution. It is the film of green fog which appears upon the surface of the negatives taken upon long-kept orthochromatic plates. To get rid of this the plate must be immersed (immediately after the hyposulphite bath is the best time for it) in a solution made of

| | |
|---|---|
| Saturated solution of hyposulphite . . | ½ ounce |
| Water . . . . . . . . . | 5 ounces |
| Saturated solution of red prussiate of potash . | 5 drops |

This solution must be prepared as wanted, and kept moving whilst the plate is in it. The effect is produced very rapidly, and the instant all fog has disappeared, the plate must be plunged into water, or reduction of density will take place. This solution is the best and safest reducer known to the writer, its action being absolutely under control, and where this process is necessary, it should be carried out after fixing, and before washing or clearing.

If, on the other hand, it be desired to intensify a somewhat thin negative, the plate, when otherwise ready for drying, is to be immersed in a saturated solution of corrosive sublimate until thoroughly bleached, say, for about five minutes, washed for a few minutes under the tap, and then plunged into a bath of solution III., or one containing about three times as much ammonia, until thoroughly blackened. It is then washed to free it from excess of ammonia, and dried.

NOTE.—As the strength of Liq. Ammon. Fort. diminishes very rapidly after the bottle is once opened, it is well to dilute a portion of it at once with an equal bulk of *distilled* water.

# CHAPTER X.

## SPECIAL OBJECTS.—I.

Very low magnifications—Illumination for them—Progressive examples—Echinus spine—Blow-fly's proboscis—*Arachnoidiscus*—Objects for high powers — *Pleurosigma* — Difficult objects — Difficulties of contrast — Excess — Deficiency — Partial dark-ground illumination — Dark-ground illumination—By transmitted light—By reflected light.

HAVING dealt in the previous chapter with the methods of photo-micrography in general, we propose to give in the present one some particulars of the modes of treatment to be adopted in the case of special objects.

As a cardinal rule, it may be said at the outset that it is always best to so arrange the relation between the aperture of the condenser and that of the objective that the angle is cut down as low as is consistent with absolute sharpness of definition of the details to be represented. Any excess in this respect leads to want of contrast, and involves the danger of a dirty background, the undesirability of which has already been pointed out.

In dealing with objects of large size, to be magnified only a few diameters, better results will probably be always obtained by the employment of ordinary photographic lenses than by the use of microscopic objectives of extremely low power. The great flatness of field in the former, and their capacity for being "stopped down," will tell strongly in their favour for magnifications up to 10 diameters. Beyond this, it will generally be found that the microscopic lenses have the advantage, since the portion of the field to be reproduced will, as a rule, be so small as to allow only the flat centre of the field to appear upon the plate.

The illumination of objects for these very low powers is a matter of considerable difficulty, since the relation between the portion of the field and the image of the radiant is in point of size a nearly equal one. With powers such as the 70 mm. objective of Zeiss, the question of aperture scarcely requires consideration, the only difficulty is to keep down the angle of the cone of rays from the condenser to a sufficient narrowness. The best plan will probably be found to be to place the radiant at least a foot from the stage, and having roughly focussed and arranged the object, to place a bull's-eye in such a position that the cone of rays which it furnishes does not cross before reaching the objective, but occupies almost the whole of its front lens. The path of the rays may be determined by their track either upon the dust of the room, or upon a puff of smoke (tobacco being usually the most convenient source) sent along the cone.

Another plan which has been recommended (by Mr. Nelson, the writer believes) is to place a plano-convex lens of long focus immediately beneath the stage, using no bull's-eye, but so arranging the lamp or other radiant as to secure even illumination.* A variation of this method, much liked by the writer, is to place a large double-convex lens (4 inches in diameter) about 3 inches below the stage.

A third plan, which gives excellent results, but is

* This plan may also be adopted where photographic lenses are to be used.

awkward to manipulate, consists in the employment of two bull's-eyes, one with its plane side close to the lamp, the other in the same relation to the object, the two being placed with their centres in the optical axis of the microscope, and their distances adjusted until the object is illuminated by an even cone of rays. By working thus, and placing a stop between the lenses, the writer has obtained some very fine results, but, as already said, the method is very difficult to work satisfactorily. With objectives of one inch focus, or less, the writer always employs the Abbe achromatic condenser, removing the front lens for powers of greater focal length than half an inch, and using it for all of greater aperture than 80°, the iris diaphragm being brought into use for adjusting intermediate differences.

Taking now a series of progressive examples, the writer recommends, as the first and easiest to be attempted, a thin section of a small Echinus spine, not more than one-eighth of an inch in diameter, and free from colour. The objective to be employed is a one-inch, without eyepiece, and the condenser may be simply the bull's-eye, or the Abbe condenser with the front removed, or other low-angled condenser, giving a cone of rays about 25° or 30° in extent. The radiant is to be placed as nearly as possible in the optic axis, about nine inches in front of the stage, the edge of the flame, if such be used, being toward the object. The condenser is next made to focus the image of the radiant in the plane of the object, and then the bull's-eye inserted and moved to and fro in the optic axis, until the circle of light described in a previous chapter is seen. The condenser is now racked up, until the light attains its maximum intensity, and the iris diaphragm gradually closed, until the whole of the details are well seen. If this be carried too far, they will become coarse in their outlines, so that several trials should be made to get the exact point. The camera being now coupled up, the size and position of the image are settled, the light read by the sensitometer, the object replaced and focussed, first upon the ground-glass and then upon the ruled screen, and the exposure made as described in the last chapter.*

Supposing the result to be successful, the next trial may be made upon the lobes of the blow-fly's proboscis, with the same objective, but at a higher magnification. The steps will be the same, but the aperture of the condenser will require to be greater, and the focussing still more exact, in order that the "zebra-markings" and the pseudo-tracheae may be well rendered.

The management of the light and object being so far mastered, a half or two-thirds inch objective may be used, and the object may be a diatom such as *Arachnoidiscus Ehrenbergii.* Here the amount of aperture to be used will be regulated by the visibility of the delicate festooned markings which occupy the interspaces between the spearheads on the inner edge of the margin.† The diatom is so nearly flat, that when the angle is reduced to the point which shows the festoons best, the whole surface of the valve is, practically, sharp, and the resulting picture is a very beautiful one.

There are few diatoms within the range of the

* In using the bull's-eye alone, the image of the radiant must be formed by it in the plane of the object, and the bull's-eye then withdrawn until the field is evenly illuminated, the lamp being used with the flame broadside-on if the image be not large enough when it is used on edge.

† The diatom must be so placed on the slide that the "ribs" are underneath, and the fenestrated membrane above.

objectives whose employment is now under consideration, which are sufficiently flat to render their reproduction easy, but *Pinnularia major* and *Aulacodiscus crux* may also be attempted with, probably, a considerable measure of success.

When we pass to consider the objects suitable for trial with objectives of a focal length of a quarter of an inch, a very much greater difficulty is experienced in selection, and the working conditions are altered by the fact that any objective which, at the present moment, would be considered respectable (or, at any rate, in the least fit for "critical" work), must have an aperture not below 100°. Such lenses are now manufactured at such a cheap rate, that most workers are provided with them, or have the means of obtaining them.

It is necessary, in the first place, to use the front of the condenser. By the consequent shortening of the focal length of the latter, the size of the illuminated area is very considerably reduced, so that very close attention must still be paid to the centring of the light, whilst, on the other hand, the image of the radiant is not sufficiently large to fill the field, so that the use of the bull's-eye is still called for.

It will always be found best in working with powers above one inch, to make the preliminary arrangements with the lower lens, and to proceed afterward to the employment of the higher objective, since the adjustments are made with very much greater difficulty with the latter lens.

With regard to objects suitable for photographic experiment with apochromatic lenses, it may be said that there are very few diatoms indeed which will not reveal their secondary structure to either a 6 mm. or a

4 mm. (=a $\frac{1}{4}$ inch or a $\frac{1}{6}$ inch), objective of this construction when used under favourable conditions. There is, however, probably no diatom which will call for more care and skill in its satisfactory reproduction than the familiar *Pleurosigma angulatum*. The fine sieve-like markings should be black, and circular, in the centre of the field. If they be white, or hexagonal, either the light is not central, or the condenser has not been properly focussed, or the objective has not been properly corrected for thickness of cover-glass, or is not exactly in focus. It is only by working one part of the apparatus against the other that the best and critical result can be secured, but the process of so doing is in itself an education. In the case of this object, very high aperture in the condenser is of much less importance than perfect correction, and a well corrected objective of N.A. .30 will give excellent results when used in place of the ordinary substage condenser, though the aperture of the objective itself may be as high as N.A. .95.

We now turn our attention to objects of special difficulty (or rather, requiring special treatment) from their individual characters.

In the first place may be considered those objects which present peculiarities in point of contrast, either between portions of the object itself, or between the object and the ground. There may be excess or deficiency in either case. Where no question of colour is involved, the difficulty of excess of contrast will be best met by giving as much exposure as can safely be allowed, and developing with a somewhat flat developer. In a case like this, very good results may be secured by the use of ferrous oxalate solution, or of hydroquinone, in fact, by either of the developers recommended for lantern-slide making (Ch. XIV.) The superior actinic transparency of the resulting negative will probably be found to assist considerably in attaining the object sought.

On the other hand, with objects which present little or no contrast, as, for example, a delicate diatom valve, the rule at the beginning of this chapter must be rigidly adhered to, and the aperture diminished as far as can possibly be done consistently with due sharpness of image.

There is yet a third class of objects under this head, small in number, but of considerable importance, which, when at their very best visual point, present portions which are brighter than any part of the illuminated background against which they are seen. In this case it is useless to attempt to obtain a white background in the print, since this can only be done by completely sacrificing the detail and fidelity of the image, and, indeed, some of the objects belonging to this class yield very beautiful prints, having the appearance of partial dark-ground illumination.

This remark suggests the possibility of obtaining similar appearances artificially, where delicate details have to be reproduced. It may be effected without difficulty, by inserting in the substage condenser a central dark stop of the size which is found by trial to yield the best results. The object in view being only the cutting out of a portion of the dioptric beam, the stop must be of much smaller size than would secure dark-ground illumination, and the substage condenser should have its aperture carefully controlled by the iris diaphragm, so that the cone of rays does not largely exceed that which the objective is able to take up. In this way the writer has been able to secure results, showing almost stereoscopic solidity, with such brilliantly transparent objects as starch-granules, in a manner which no other method has yielded in his hands.

From this partial darkening of the ground we naturally pass on to consider the question of black-ground illumination, in which the object does, or should, appear brightly illuminated upon a ground of velvety blackness. The method may, with proper care, and suitable appliances, be used with objectives of very considerable N.A., but it finds its chief application among those whose aperture does not exceed N.A. 0.40, or, *roughly speaking*, with powers up to one-third of an inch in focal length. By this plan, the object aimed at is the complete cutting out of the *transmitted* beam from the condenser, only those rays being allowed to enter the objective which are *reflected* from some portion of the object itself.

A very beautiful example of this kind will be found in the appearances of minute *Foraminifera*, mounted dry, and viewed under a one-inch objective. The condenser, bull's-eye, object, and objective, having been all brought carefully into the optic axis (this being a matter of very considerable import to our present purpose), a central stop is inserted into the back of the condenser. The stop must be of such a size that when it is inserted, and the back lens of the objective examined by removing the eyepiece, only a faint glimmer of light is perceptible. Upon the other hand the stop must not be larger than is required to effect this object, or a very great loss of valuable light will ensue. The object being now examined, it will be found, if the stop have been selected on the above lines, that the objects themselves are brilliantly illuminated, and stand out in high relief upon a beautifully soft, velvety background, every detail of their surface markings being very clearly shown up. A certain amount of "flare" is very likely to be seen round the object. This will be found to be due, either to incorrect focussing of the condenser (to mention which is to indicate the remedy) or to excess of aperture, involving the use of the iris diaphragm to narrow the cone, or perhaps to a combination of these two causes. If the flare be confined to one side of the object, the condenser is not centred, or the bull's-eye, or radiant, is not in the optic axis, and in this case, too, the cause must be hunted down and eliminated.

By adopting the foregoing plan, the secondary markings on *Triceratium favus* may be shown without difficulty with a $\frac{2}{3}$-inch or $\frac{1}{2}$-inch lens, and Mr. Nelson has succeeded in doing it with a one-inch objective. There is no difference in the method, the only thing which need be said is, that still more scrupulous care must be taken in making the adjustments. The methods above described will be found so thoroughly satisfactory that there is no need to touch upon the use of Wenham's paraboloid, or the spot-lens. Both had their day; the former, in particular, is a most beautiful and ingenious application of a great mathematical principle; but in both cases the light was stopped out as it *left* the condenser, and it is now a matter of universal agreement that the *lower* end of the condenser is the proper place for the stop.

The method of dark-ground illumination just described is of course only practicable with objects of a transparent nature. With opaque objects it is necessary to throw the light upon them from above the stage, and the exposures, being made by reflected light alone, are of considerable duration, whilst the necessity of sending the light in between the object and the objective limits the method to low-power work, where the working distance of the objective is considerable. The lighting, in this case, is best accomplished by using one bull's-eye to parallelise the rays from the lamp, and a second one to concentrate the parallel rays upon the object. Some difficulty will be found in obtaining a visible image upon the ground-glass screen, and it is therefore advisable to work in a room which is entirely dark. There is no special difficulty in focussing. Very beautiful results may be obtained with such objects as *Foraminifera* by the above plan.

In photographing objects with the lowest powers the roundness of field of nearly all such lenses occasions a good deal of trouble. To obviate this the writer contrived the double bull's-eye arrangement previously referred to. He now finds it better to place an adapter, in which is a small iris diaphragm, upon the microscope tube, and to screw the objective on to the adapter. By closing the diaphragm the sphericity of the field may be regulated as required.

On the question of contrast it may be noted that when this is weak, a slow and thickly-coated plate should be used, and conversely; and in violent contrasts it will be found advisable, in addition, to develop with "Rodinal," which gives much detail, but yields distinctly thin negatives.

# CHAPTER XI.

## SPECIAL OBJECTS.—II.

*Colour difficulties*—Orthochromatic plates—Their preparation—Their use—Difficulties at various points of the spectrum—Use of coloured screens—*Difficulties of form*—Low *v.* high aperture—The writer's method of successive exposures.

THE extent to which the difficulty attending the reproduction of coloured objects by photographic means has been overcome is one of the most marked features in the progress of the art; especially is this the case with reference to photo-micrographic work. In photographing objects in the usual way, by reflected light, the formation of the image is largely due to the effect of rays of all colours reflected by all parts of the object.* In the formation of the microscopic image, on the other hand, the light which impresses the plate has been filtered by the object, and it very frequently happens that, with objects of certain colours, the whole of the actinic rays, commonly so-called, and even those far nearer the red end of the spectrum, in part, are absorbed in their passage, so that hours of exposure would fail to produce any effect even upon the most sensitive plate unless the latter were specially pre-

* To such an extent does this take place that it has been calculated that if the moon were covered with black velvet, her brightness would be practically undiminished.

pared. To this must be added the undoubted fact that the rays at the red end tend to undo the work done by those at the violet end, so that if the latter be feeble and the former strong, the difficulties of reproduction may be greatly increased in consequence. These difficulties are very much lessened, though not by any means removed, by the use of orthochromatic plates, which, when properly prepared and used, have the power of so responding to the vibrations of light of various wave-lengths as to reproduce the *luminous* impression conveyed by vibrations of the same length when they impinge upon the retina. The method of preparation consists in the employment of an aniline dye, usually one of the fluorescin compounds, in a solution of which, with or without ammonia, the plate is bathed, and subsequently drained and dried.

Mr. Bothamley has given two formulæ, one for staining plates with erythrosin, the other for effecting the same process with cyanin. They are as follows :—

| | |
|---|---|
| Solution of erythrosin B.* (1 in 1000) . . | 1 part |
| Solution of ammonia (1 to 10) . . . . | 1 ,, |
| Water . . . . . . . . . | 8 parts |

The plates are first soaked in a solution of ammonia (1 part of Liq. Ammon. Fort. to 99 of water) for two or three minutes, then immersed in the dye solution, drained, and dried in the dark. The whole of the operations must be conducted in deep ruby light and the plate shaded. The cyanin solution † is made up of

* Of the Badischer Anilin und Soda Fabrik.

† It seems desirable to note that Messrs. Edwards & Co. claim that the use of ammoniacal solutions of fluorescin compounds is protected by their patent in this country. As, however, the dyes above mentioned do not correspond with the characters of those described in the patent (though the effect is the same) it is doubtful whether they would think it worth while to interfere with the use of them by amateurs.

| | |
|---|---|
| Cyanin solution (1 in 500) . . . . . | 10 parts |
| Strong ammonia . . . . . . . . | 2–4 ,, |
| Absolute alcohol . . . . . . . | 10 ,, |
| Water . . . . . . . . | 200 ,, |

In staining plates with cyanin, the operations must all be conducted in what is practically absolute darkness.

Upon the whole, the amateur, who only needs a few plates now and then, will probably find it preferable to purchase those few ready stained. Under any circumstances the bathed plates do not keep well, and after a time the result of pyro-development is apt to be a film of green fog with a metallic lustre, which has to be removed by the use of a reducing solution, involving a certain loss of density.* Bought, or home-made, the plates so prepared are of enormous advantage in dealing with coloured objects.

It will, perhaps, be easiest and most systematic if we follow the order of the spectrum in dealing with the colours which may present themselves. We naturally, then, begin with violet. Under this heading we have hæmatoxylin and the anilin violets, the former inclining as a rule to blue, and the latter to red. Even with orthochromatic plates violet-stained objects present but little contrast to the uncoloured background, when the oxy-hydrogen light is used, the latter being rich in those rays which occupy the violet end of the spectrum. Since, however, the whole of these pass through the field, and some of them are arrested by the object, the use of a screen of pale yellow glass, or glass coated with a solution of aurantia (an aniline dye) in collodion, has much less effect upon

* With other developers this film does not form ; it appears to be due to the presence of the ammonia in the pyro-developer.

the field than upon the object, the photographic contrast of the latter with the field is so raised as to yield a highly successful photograph.* The same remarks will apply to blue, but of all colours the most difficult to render satisfactorily is this particular one when the object is only faintly stained with it, and the writer confesses that he has been occasioned far more trouble by objects so stained, or rather not stained, than by those of all the other colours of the spectrum put together. At the same time, it must also be confessed that the practice thus gained has done much to facilitate his work with less obnoxious dyes. The blue region once passed, the difficulties for a time diminish. A yellow object, with or without a pale yellow screen, or even an orange-coloured one under the same conditions, will seldom offer any considerable difficulty, though the use of the screen will probably be imperative with the latter colour, in order to secure satisfactory detail in the lighter portions of the object.

We are now, however, called upon to deal with a class of objects in which the most violent contrasts of all are to be found, in which we have to represent, at the same time, perfectly clear structures and dense brown ones, and to preserve the detail in both. Next to faintly stained blue preparations, the colours of the insect world will call for the greatest exercise of skill and patience upon the part of the photographer. This applies especially to displayed insects. The writer well remembers his struggles with a "yellow streaked frog-hopper," an insect with clear wings, delicate pointed antennæ, and a dense brown body, upon the centre of which were somewhat lighter markings,

* With lamp-light, well-stained violet preparations may be photographed without the use of a yellow screen.

resembling a "death's head." He finally succeeded in reproducing it with fairly equal justice to its various parts by the use of a deep orange screen of Warnerke's orthochromatic glass (as used in the earliest days of orthochromatic photography), with an exposure to lamp-light of several hours' duration, under a magnification of about 25 diameters! The exposure would doubtless be shorter now, but the method of treatment would of necessity be the same. A pale yellow screen would be absolutely useless. Even the orange screen failed him in one instance, the object being a marine worm, with black eyes, brown jaws, clear *setæ*, and a body heavily stained with carmine. The problem was to show the eyes and the darker staining of the digestive canal, whilst preserving the appearance of the *setæ*. In this case, a deep ruby screen was resorted to, and with the happiest results, though here too, in spite of the low magnification, the exposure was enormously long. The above practical instances will probably show, better than a purely didactic discussion, the way in which these colour difficulties must be met. The screen must be so chosen as to obstruct the coloured rays to such an extent as to reduce the lighter portions of the object, since the rays passing through them are very much more reduced, relatively, by the screen than are those rays which the darker portion allows to pass. We shall have to consider, under the head of the photography of Bacteria, the method of dealing with dense masses of organisms, in a preparation which is thin in other respects.

The difficulties arising from the form of the object to be represented are less easily overcome than any of those which have been previously dealt with in this chapter, and every method of overcoming them has its own disadvantages.

Objects which present at more than one level features requiring reproduction, may be dealt with in one of two ways, each of which has its own limits, these being somewhat narrow ones.

The quality of "penetration" of any object glass stands in *inverse* proportion to its angular aperture, upon which again depends its definition and resolution, these two *directly*. The greater the aperture, the nearer to a mathematical plane, or surface, does the field of view become, as regards its depth. On the other hand, narrowness of aperture carries with it an increase of penetration, that is to say, the more nearly parallel the bounding rays of the cone which enters an objective, the more nearly are they brought to a focus at the same point by that objective.

Thus, with slightly concave, convex, or irregular objects, the cutting down of the aperture, or better still, the employment of a low-angled objective, may suffice to overcome the difficulties they present. The reason for preferring the low-angled objective is that we have to consider not only the cone of rays transmitted by the condenser to the objective, but also the fact that these rays are varied as to their direction by their passage through the object, and may be so refracted by its various parts as to utilise that portion of the aperture of a wide-angled objective which is supposed to be cut off by stopping down the condenser.* With an objective of low aperture these rays are not admitted at all, and hence the resulting image is much clearer and sharper than that yielded

* So far as the writer is aware this point has not been previously raised, but it is one of great practical importance.

by a stopped-down objective of higher aperture, though the "penetration" is the same.

With such objects as *Craspedodiscus*, which requires a fairly high power for its delineation, and has details lying in at least three planes, the only possible method of securing a fair representation is to focus down upon each successively. To effect this satisfactorily, good mechanism in the fine adjustment, and great care on the part of the operator, will be indispensable. The writer has usually proceeded by first of all arranging the position, magnification, and time of exposure, and then determining the several planes to be reproduced. The time of exposure required for the whole object is doubled, and this doubled period is divided between the number of exposures to be made, allotting one to each plane. The greatest care must be taken that the fine adjustment does not cause the image to traverse, or the several impressions will fail to occupy their relative positions. In all but the very worst fine adjustments, some point will be found which can be relied upon in this particular.

The plane which lies uppermost is focussed, the dark slide (in which the plate must not be able to shift) is inserted, the previously determined portion of the exposure given, the slide lowered, the plate removed with the utmost gentleness, and the next plane focussed and exposed for, and so on, until all have been done.

It must be remembered that the plate as a whole will be over-exposed, and should therefore be treated, as recommended on page 108, for objects presenting violent contrasts of density. In any case, the resulting negative will be somewhat flat, but, if properly managed, all the details will be there, and this is after all the chief object of a photograph.

Objects such as *Campylodiscus* do not lend themselves to any method of reproduction by photography, and must be regarded as being at present beyond its sphere of operation.

# CHAPTER XII.

## BACTERIA.—PHOTO-MODELLING.

Unstained bacteria—Stains for bacteria—Photographing flagella—Focussing bacterial images—Organisms in pus, blood, &c.—Stained grounds—Organisms in dense clusters—Sections—Bacterial—Embryological—Photo-modelling—Methods of reproducing form from photographed sections.

The rapid strides which have been made by the science of Bacteriology, and the extreme difficulty and importance of registering with absolute fidelity the appearances presented by the organisms with which it deals, coupled with the comparative ease by which this object can be attained by photo-micrographic processes, especially since the introduction of apochromatic lenses, seem to furnish sufficient justification for the devotion of a chapter of this work to the subject. As a practical bacteriologist the writer has often been struck by the remarkable differences exhibited between living organisms and the same ones when dead, dried, and stained. The cover-glass preparation is no doubt a necessity of the work, and furnishes information available in no other way, but the writer contends strongly that for complete registration of results it is necessary that far more attention should be paid to the photography of bacteria in the living condition, or as nearly so as possible. Highly motile organisms will no doubt present difficulties, but the exposure of the drop of fluid on the cover-glass to the vapour of osmic acid for a few seconds will kill and fix the organisms without altering their size or form, whilst if the fluid be in a thin layer, there will be no movement of the organisms such as to call for the employment of a vertical apparatus.

It is unnecessary to say that, to obtain successful results in work of this character, very careful arrangement of the light is required, and it may be advisable to have recourse to the method previously referred to of using a central stop, of small size relatively to the aperture of the condenser, so as to cut out the centre of the dioptric beam, and obtain partial dark-ground illumination. The writer has, however, usually adopted the method of closing the iris diaphragm of the condenser, and so obtaining contrast. The latter must, in consequence of the want of colour, and the small margin between the refractive indices of the organisms and of the fluid in which they float, always be feeble. The result will naturally be that the negatives must not be at all over-exposed, or the contrast will be altogether lost, and in any case the resulting prints cannot be expected to have a pure white background, though very fairly satisfactory lantern slides may be obtained.

The cases in which permanent preparations of unstained organisms may be made are perhaps few in number,* and the method of drying upon the cover-glass, with subsequent staining, cannot be dispensed

* Probably a good deal more may be done in this way than has yet been attempted. The writer fancies that by diluting the culture with salt solution and exposing a minute drop upon a cover-glass to the vapour of osmic acid, subsequently mounting simply by placing the cover-glass upon a slide and "ringing" with Hollis's glue, very many species may be permanently preserved in the natural condition.

with.* It is only necessary to remark here that gentian or methyl violet will be found at once the most precise and most satisfactory of all stains. Speaking from a considerable experience, the writer has no hesitation in saying that half the uncertainty of bacterial photo-micrography is due to the over-staining and necessarily *somewhat* imperfect decolorisation to which it is the custom to subject preparations. Given a good sharp stain, the photography of bacteria at 1000 diameters, on orthochromatic plates, presents no greater difficulties than the reproduction of an echinus spine at 50 or 100 diameters.

The most difficult of all bacterial photographic exercises, in fact the most extreme test of any worker's skill, is the reproduction of the flagella of motile organisms, and in the writer's opinion (in which he is supported by the experience and public statements of his friend, Mr. Pringle), the most difficult test diatoms are more easily to be photographed than the flagella of such an organism as the *Spirillum Choleræ Asiaticæ*. The fine adjustment must be absolutely steady and dependable, the temperature constant, and the worker's power of accurate focussing highly developed in order to produce a respectable, to say nothing of a first-rate, photograph of such an object.

It should have been stated, in connection with the remarks upon cover-glass preparations, that the focussing requires special attention, for the reason that the organisms are cylindrical or spherical in form. When the objective is above the focus, the images appear brighter, and when it is below the focus, darker than they do when exactly in focus. The

* The writer has endeavoured, in the Appendix, to indicate the methods which he has found most satisfactory.

darkness of colour is therefore not a reliable guide, and the writer's plan is to begin above the focus, and work down until the bright line or spot which the image at first exhibits *has just disappeared*, and *at that point to stop.* The image is then left whilst the plate is being put into the slide, the light (oxy-hydrogen in the writer's case) left burning, and the image again examined before inserting the plate. If the slightest falling off is perceptible, the focussing process is repeated, and the writer prefers to spend half an hour over the focussing rather than inevitably to waste plates on an unsatisfactory image.

Still more important, perhaps, than the photography of pure cultivations of bacteria is that of the organisms as they occur in disease, as, for instance, in blood, pus, or other fluids or secretions, and in solid organs or tissues.

When occurring in fluids, the organisms will usually have to be photographed from spread, often double-stained, preparations, and here difficulties will arise from the fact that the ground is seldom free from stain, and has often taken the dye to a considerable extent. If the organisms be stained with methyl blue and the background with eosin or rubin, the photo-micrographer has a pleasant task before him, speaking ironically! His best chance in such a case is to use as dark a yellow screen as will allow of a not unduly prolonged exposure, in the hope of lessening the actinic transparency of the organisms whilst leaving that of the background unaffected. It is not possible to give instructions which will meet every case, but the foregoing sentence embodies the only principle upon which success can be hoped for in any measure.

Another difficulty which is met with in such spread preparations, arises from the fact that the organisms are often in dense clusters, presenting a mass of dye, and that in such cases the decolorisation is insufficient as regards the clusters, or else overdone as regards scattered organisms in the same field.

In either case the resulting contrast is excessive, and the plan to be recommended for adoption is that of exposing sufficiently to get some detail in the cluster, and subsequently developing with a solution which tends to flatness, such as ferrous oxalate or "Rodinal."

It is but rarely that either these negatives, or those of unstained organisms, will yield satisfactory bromide prints. Aristotype paper is a far better medium, and a lantern-slide gives the best results of all.

Proceeding now to the consideration of sections, pure and simple, the first difficulty is that even in the thinnest of them, the objects do not lie in one plane, whilst from the high magnification which is practically inseparable from photographic reproduction of them with their contained organisms, the limiting planes are by magnification widely separated, and yet, for the same reason, neither of the methods previously recommended (p. 118), in such cases is likely to be successful. Two distinct methods of treatment have been adopted. Dr. Neuhauss, the quality of whose work is beyond challenge, advocates the closing of the condenser to about one-third the aperture of the objective, thus bringing the planes together. Mr. Pringle, on the other hand, uses the full aperture of the condenser, so as to exclude, as far as may be, all planes but one. The writer has used both plans, and has succeeded, and failed, with both. Where the organisms are long, and lie obliquely to the plane of the section, the use of the low aperture will probably give the best results ; where they are more spherical, or of very small dimensions, the higher aperture will give clearer and more satisfactory images. The greatest difficulty of all will be found in photographing organisms within cells, and here no plan can be absolutely relied upon to meet every case. The writer has sometimes found it necessary to use his condenser as an "oil immersion" one, and at others to cut down the aperture even below the limit assigned by Dr. Neuhauss.

The bacteriologist who has photographic reproduction of his results in his mind, will do well to cut his sections as thin as possible. Some notable opponents of "gossamer sections" have been led, by practical experience, to abjure their opinions, and the writer has always held that sections for this work cannot be too thin. On the other hand, for general biological work, sections of from 1–2500 inch to 1–3300 inch in thickness will, according to the writer's experience, furnish the best results, with magnifications up to 400 or 500 diameters, delicate embryological specimens being specially unsuitable for photography when thinly cut.

For photo-modelling, which is capable of rendering very great service in the study of zoological objects, too small for dissection, the writer recommends the following method of procedure. The objects should be cut in paraffin, on an automatic microtome, in ribbons, mounted serially on slides and photographed separately, at *one and the same* magnification. The resulting prints are then mounted on cardboard or thin wood, which represents, with the print upon it, the same magnification in thickness which the print shows in area. The outline of the prints being then cut out with a fine saw, it is evident that the whole, when placed in order, will give the contour of the object, whilst the separate prints will show the detail at corresponding points of the length or breadth as the case may be.

The best results will perhaps, indeed probably, be obtained by cutting the sections at about 1–2500 inch, and photographing every fifth one, in which case the mount should of course represent five times the thickness of the magnified section. It is scarcely probable that a well-stained section, 1–500 inch in thickness, could be successfully photographed.

The above plan is based upon the one to be found in Bolles Lee's "Microtomist's Vade Mecum," but the method there described involves an elaborate system of drawing by means of ruled squares, in the eyepiece and upon the paper. No more striking instance of the superiority of the camera over hand-work could possibly be given.

## CHAPTER XIII.

### PRODUCTION OF THE POSITIVE.

Printing-out—Materials—Toning—Fixing—Washing—Glazing—Choice of a method — Printing by development — Eastman paper — Alpha paper — The new platinum process.

THE great value of photo-micrography depending upon the fact that it yields results free from bias, it is a universally accepted canon that retouching, farther than the "spotting out" of unavoidable pinholes in negatives, is inadmissible. A good print will tell the truth, and nothing but the truth, though optical laws may prevent it from telling the whole truth in more than one plane.

The present chapter will be devoted to details connected with the reproduction of the original object in its true aspects of light and shade, which are, of course, reversed in the negative.

First, in point of chronological order and, as the writer thinks, in point of general excellence and faithfulness, stands the old-fashioned silver print, made by placing a piece of sensitised, albumen-coated paper, under the negative, in contact with the film, in a printing frame, and exposing it to daylight. The transparency, even in the darkest shadows, and the general softness of the resulting print give effects of the highest artistic value, which in some respects are not approached by any of the processes in which a black tone is produced by development of the print with iron or other salts.

Under this head, and as meriting the same praise, are included the processes in which the negative image is "printed-out" upon gelatine or other films containing chloride of silver, among which Liesegang's Aristotype paper, and Eastman's "printing-out paper," must have the first place accorded to them. Probably no other process in existence yields such exquisitely beautiful results as a good print upon one of these media, glazed by drying it upon vulcanite or upon plate glass rubbed with talc. Directions being enclosed with each packet of the paper, it is unnecessary to say very much about the process itself; it does not differ materially from the one now to be described for producing albumen prints.

The negative being laid, face upward, in an open printing frame, the sensitised paper is laid upon it, face downwards, and the back carefully put in so that it can be opened again without causing the paper or negative to shift. This is essential, for the print must be examined during the printing process, and the least alteration will ruin the print. The exposure is made by laying the frame with its face to the sky in a place sheltered from the direct rays of the sun, and the action of the light is allowed to continue until the print is at least somewhat—it may be considerably—darker than it is desired to have it when finished. A good deal of density is lost, especially by weak prints, in the subsequent processes. The print being sufficiently exposed, it is removed from the frame, the name written upon the back with lead pencil (working on a hard surface with a soft pencil) if required, and then either finished as below, or put aside for a day or two whilst others are accumulating.

The print, or prints, are finished as follows. The first step is to wash out the excess of silver. The prints are placed in a dish, and either kept revolving by a constant stream of water, or washed by moving them to and fro in water, until all milkiness of the water has disappeared. This, as well as the subsequent steps, must be undertaken in very subdued daylight, or by artificial light, or the prints will be discoloured. The washing completed, the toning process follows. Any number of toning baths may be found in works on photography, but one which satisfies the writer's aspirations completely, and which he strongly recommends, is Werge's borax bath, made up of

| | |
|---|---|
| Borax solution (1–60) . . . . . . . | q.s. |
| Chloride of gold . . | 1 grain to 8 ounces of above. |

The bath is ready for use at once, and by adding more gold to it when required, may be employed for some time, though it works best when fresh.

In the toning bath the prints remain, with constant stirring, until the foxy red of the washed print has entirely disappeared, and given place to a beautiful brown or purplish tone. If any red be left, it will be rapidly attacked during the next process.

The prints, after being rinsed in water, are ready for fixing, and this is effected in a hyposulphite bath of the same strength as that used for negative work, and occupies about ten minutes. In all the above processes care must be taken to have sufficient fluid to allow the prints to move freely, or the results will not be satisfactory.

The final washing will take about six hours, and the period cannot be greatly shortened without much risk of subsequent fading.

Negatives which present violent contrasts will often yield better prints by direct sunlight. The rapid action of the light upon the upper layers of the film in the lighter parts protects the lower ones during the time that the details in the darker parts take to print properly. On the other hand, very thin negatives are best printed by very feeble daylight.

The above process is also applicable to emulsion papers, and Werge's toning bath yields excellent results. The printing must not be carried too far.

Prints made by either process may with advantage be glazed. In the case of Aristotype prints this may be effected by "squeegeeing" the print on to polished vulcanite, or on to plate glass which has been well rubbed all over with a cotton-wool plug loaded with powdered talc, which is subsequently cleaned off with a handkerchief until the glass looks quite free from it. Albumen prints may be glazed in a similar way, but in both this and the former case the gloss will disappear in mounting. This may to a great extent be overcome by brushing over the back of the prints when dry with a solution of mastic ($\frac{1}{2}$ oz. to one pint of methylated spirit). The best way of all is to mount the prints whilst on the glazing surface, and still moist, by brushing starch paste over the back of them, and pressing on to them damp cards somewhat larger than required for the finished mount. The whole is then allowed to dry, and having become so the prints can be peeled off with the greatest facility and have a highly polished surface. Prints may also be burnished or enamelled after mounting, but these processes belong to the domain of the professional photographer rather than to that of the scientific student.

The next class of prints with which we have to deal is that in which the image is impressed upon prepared paper by means of exposure, relatively very short, to day or artificial light, but requires to be subsequently developed in the same way as a negative.

The processes themselves, as regards the details of development, need hardly be referred to here. The makers furnish full details in every packet of material, and the mere reproduction of them would be of little service. As to the results obtained, they may be divided into two classes, in one of which, represented by the Eastman permanent bromide paper, and other bromide papers upon the same lines, a black and white print is the object aimed at, whilst in the other a more or less successful attempt is made to obtain by a combined process of development and toning the same tints as are associated with ordinary silver prints done by printing-out processes. The well-known Alpha paper is of this class.

For general photo-micrographic work the writer considers that the Eastman bromide paper is likely to hold its own against all rivals for a long time to come. He has made thousands of prints upon it, and whilst it requires considerable care in manipulation, and is very sensitive to variations in length of exposure and strength of developer, the general results are admirable. Microscopic objects are, however, but very exceptionally black and white, and the black of Eastman and other bromide papers is so very black, that detail in the shadows is difficult to obtain, and there is a great lack of the transparency which lends so great a charm to good albumen or Aristotype prints. Again, weak negatives, and those presenting violent contrasts, both of which may be made to yield passable results by printing-out, do not lend themselves readily to reproduction in black and white. With a good clear negative, crisp and plucky, the bromide papers give prints closely resembling fine engravings, and it is in negatives of this class that the process finds its chief employment, and shows to the best advantage.

One point in particular tells strongly both for and against bromide paper. It has already been said that a good picture on a clear white ground is the "critical image" aspect of a photo-micrograph. In printing-out, the ground may, and indeed should, be allowed to become slightly affected, since there will be a considerable loss of density in subsequent processes. In bromide printing, there being no loss of density, and reduction being practically impossible, if the ground be in the least degree printed, the print takes on and retains a disagreeable smoky aspect which is, to say the least of it, extremely inartistic. In fact, neither bromide prints nor silver (*i.e.*, printed-out) prints can be dispensed with, but each process has its applications and its limits.

Of the combined development and toning process required by the Alpha paper the writer has little experience; judging from what he has seen he is inclined to think it might be made to answer very well, but it is not easy to obtain anything like a uniform tone in a number of prints.

Of the platinotype process in its various modifications his experience is still smaller, but of this too he has seen very fine specimens, notably in the hands of Mr. Pringle, who has used it largely.

That gentleman is, however, of opinion that it is distinctly inferior to bromide printing for general photo-micrographic work. The new cold-bath process seems likely to form a distinguished exception to the above statement, and the writer has seen some very fine specimens of work done by it.

It may be added here that when a number of prints from the same negative, and absolutely alike, have to be produced by any process involving development, not only must the exposure be the same for each one, but every print must be developed with fresh developer. This may of course be done by developing a number of prints together, which is probably the least troublesome plan.

No developing solution known to the writer will yield identical results with two successive prints (or negatives) similarly exposed, the speed of development diminishing and the amount of contrast increasing each time it is used. In fact, when over-exposure has to be allowed in order to bring up detail, it is often very advisable to use a developing solution which has been previously employed.

## CHAPTER XIV.

### LANTERN-SLIDE MAKING.

Advantages of lantern slide—Choice of plates—Tones—Exposure—Development—Fixing—Washing—Clearing—Drying—Mounting and adjusting.

THE impossibility of exhibiting microscopic specimens to a large number of persons simultaneously, and the general increase of interest in microscopic and especially bacteriological subjects, have led to a large extension of the use of the optical lantern, by means of which photographs may be exhibited to a large audience under very favourable conditions.

The preparation of lantern slides for this purpose is one of the most delightful occupations of the photo-micrographer; and the reproduction, as regards transparency especially, of the conditions under which the negative was taken, renders the representation so obtained the most faithful of all. In some respects, too, the process is the easiest of all to work, and the photo-micrographer is strongly recommended to familiarise himself with the details of it.

A whole host of lantern plates may be found advertised in the journals, but, desiring to speak well of the bridge which has carried him safe over, the writer recommends, and always uses, Thomas' lantern plates; and has found them equal to all his varied requirements. They are slow, which is a very decided advantage, and in a long experience of them the writer has only once or twice known them to fail. There are, however, many other kinds which yield excellent results.

A great variety of tones may be obtained by various methods of treatment; but for our present purpose the cold or black tones are by far the most suitable, and will alone be dealt with.

To make a lantern slide, then, the lantern plate is put on to a negative in the printing frame, the two films being in contact. The surface which is coated is not very easily distinguished by a novice, but a very simple method will decide it. If the glass side be breathed upon, it will become misty, if the same be done upon the film side, no cloud forms. It is necessary to be careful that the centre of the picture occupies the centre of the lantern slide, or the result in the lantern may be unsatisfactory.

Both negative and plate should be most scrupulously brushed with the "softener" before being placed in contact. A lantern slide is intended to be magnified, and it becomes therefore doubly desirable to eliminate all avoidable blemishes.

The exposure is most conveniently made by the light of a paraffin lamp, or a regulator burner, and with a Thomas' plate will vary from three seconds to as many minutes or more at one foot from a 1½ inch "Paragon" lamp, with a flame two inches high, placed one foot away from the printing frame.

The developers suitable for the purpose of bringing out the image are numerous. The writer uses the same solutions as for pyro-development of negatives, but the proportions are altered. To make an ounce of developer *equal parts* of solutions I., II., and III., and of water (2 drachms of each) are required. The exposed plate is taken out of the frame, by yellow light, in the dark room. It is placed face upwards in a dish, soaked in water for a minute or two; the water is then poured off, and quickly replaced by the developer. The image appears more slowly than in the case of negative work, and time must be allowed for this. If, at the end of one minute, the margin is fairly started* and the image is just showing up, the exposure is probably correct, and all that is required is to rock the dish until the desired density has been obtained. In this point practice alone can guide to certainly satisfactory results.

The usual fault is that from fear of spoiling the appearance of the ground either too little exposure is given or development is arrested too soon. It must be remembered that lantern plates lose density very considerably during the fixing process, and that provided the ground only very slightly loses its whiteness, there will be no trace of veiling in the finished plate. Even supposing the ground not to be absolutely clear at last, the fault, though no doubt a serious one from a technical point of view, is yet venial as compared with a washed-out-looking image.

In printing, by whatever process, it is absolutely necessary to give sufficient exposure to impress all details upon the paper or plate. If they are not impressed by the action of the light, no amount of development will bring them up, and the only result of forcing the development will be to spoil the clearness of the ground without materially improving

* The above criterion is, of course, only available when a mask has been used in the dark slide, or, at all events, somewhere in front of the negative, during the production of the image upon the latter.

the image. Under-exposure is a more serious fault than the reverse, though it is hardly necessary to say that both are better avoided.

Supposing the image to be distinctly apparent upon the back of the plate, and to appear as dark by transmitted light as a woodcut on white paper would under the same conditions, it may be concluded that the result will not be far off the mark, and the slide may now be thoroughly washed and immersed in the hyposulphite bath. Here it is to remain for at least five minutes, and is then to be thoroughly washed, and after removal of the hypo. is to be immersed for a few seconds (10 to 60) in Edwards' clearing solution, composed of

| | |
|---|---|
| Ferrous sulphate . . . . . . . | 3 ounces |
| Sulphuric acid . . . . . . . | 2 drachms |
| Alum . . . . . . . . . | 1 ounce |
| Water . . . . . . . . . | 1 pint |

If over dense, reduction may be attempted by allowing the plates to remain longer in this solution, but the writer's experience of reducing lantern plates is that the details go before the density is materially affected, and he does not advise it, for this reason.

A thin slide may be intensified in the same way as a thin negative, by immersion, after thorough washing, in a solution of bichloride of mercury (corrosive sublimate), washing to remove excess of the reagent, and blackening with ammonia, followed by more washing. The only circumstances, however, under which this plan is allowable, are when a slide has been over-exposed, and the development arrested in consequence when all detail was out but density still wanting. Detail cannot be *obtained* by intensification, any more than by forced development. In fact, it may be repeated, as a universal rule, in all developmental processes, whether negative or positive, that detail and exposure stand in the most intimate relation to each other.

The developer above referred to is the one which the writer prefers, for many reasons. In the first place, it gives good brown-black tones, which show up well upon the screen. In the second place, it is cheap and keeps well, and moreover, its use obviates the necessity for having a whole host of bottles in the dark room. In the third place, it is very manageable, and slight variations in the proportions of bromide and ammonia produce considerable results upon the quality of the slide, increase of bromide tending to more density, increase of ammonia to greater fulness of detail—in other words, bromide for pluck, ammonia for flatness. This quality is of very considerable value, when a slide with violent contrasts has to be printed. A fairly full exposure, with a developer slightly increased in ammoniacal strength, conjoined with the transparency of the slide, will enable the operator to reproduce the details of both the denser and the thinner portions of the negative.

In this matter of transparency, the lantern slide has a very distinct advantage over every reproduction of the microscopic image, since the field of view of the microscope is rendered as nearly as possible under its original conditions, in fact, there is an air of verisimilitude about a well-projected image such as is to be found nowhere else but in the microscopic image itself.

It would scarcely be appropriate to leave this very important part of our subject, without mentioning the other chief developers which may be employed for producing lantern slides.

Next to the pyro-developer, the hydroquinone developer certainly must have a position allotted to it. It gives images of a most beautiful black tone, and were it dependable, in point of keeping qualities, would alone suffice for all lantern slide work. Unfortunately, neither the hydroquinone, nor the sulphite of soda, which is an essential ingredient of every developer containing hydroquinone, can be relied upon to maintain its efficiency, either dry or in a state of solution, alone or combined, at any rate for more than a limited period, one of far less duration than that required to perceptibly affect the efficiency of pyro. and metabisulphite of potassium when dissolved together.* The reader will certainly do well to give hydroquinone a trial, and where the solutions are not required to be kept very long, he will not wish for a better developer, but where a lantern slide has only to be made now and then, the slight difference in quality of image does not compensate for the trouble of making fresh solutions, which, owing to alteration in the chemicals employed, may turn out to be useless after all. Messrs. Thomas issue with their lantern plates a most excellent formula for a hydroquinone developer. It is as follows:

| | | |
|---|---|---|
| 1. | Hydroquinone | 160 grains |
| | Sodium sulphite | 2 ounces |
| | Citric acid | 60 grains |
| | Potassic bromide | 40 " |
| | Water | to 20 ounces |
| 2. | Sodium hydrate | 160 grains |
| | Water | to 20 ounces |

For a developer take 1 part each of solutions 1 and

* For some reason the metabisulphite does not act very satisfactorily in conjunction with hydroquinone.

2, and 2 parts of water. The results, with proper exposure, are certainly not excelled by any other developer.

A third developer of the same class is the new "Rodinal," the simplest of all, since the developing fluid is made by merely adding 15 drops of "Rodinal" to one ounce of water. In the writer's experience, however, the lantern slides produced by Rodinal are apt to be thin and flat, and he cannot very strongly recommend it. On the other hand, the very thing which is against it as a lantern-slide developer is in its favour as a developer for bromide paper, and for this purpose he has used it largely and with considerable satisfaction.

In a class by itself stands the well-known ferrous oxalate developer, which covers precisely the same ground as hydroquinone, produces almost identical results, and gives perhaps slightly clearer lantern slides, upon the whole, than any other process. In fact, the writer never decides, before actually beginning work, whether he will use the pyro. or the iron developer, and the final factor is usually the one of convenience at the moment. The proportions of the developer are as follows:

| | |
|---|---|
| Solution I. (for pyro-developer) . . . | ½ drachm |
| Saturated solution of neutral oxalate of potash | 9 drachms |
| ,, ,, ferrous sulphate . | 1½ ,, |

It is usually asserted that it is absolutely necessary to mix the solutions in the order above given. The writer finds that although a temporary turbidity may sometimes ensue on adding the oxalate to the iron (instead of *vice versâ*), this is more than counterbalanced by the convenience of measuring the smaller quantities in the lower and smaller divisions of the measure ; the precipitate dissolves again immediately.

The ferrous oxalate developer must be acid when used, and it is therefore desirable to acidify the iron solution with sulphuric acid (without which, indeed, a clear solution can scarcely be obtained) and the oxalate of potash solution with oxalic acid, before they are used. An alkaline developer will give rise to mealy-looking lantern slides.

Of all the above developers, it may be said that they may be used for more than one plate. The second will probably develop as well as the first, the third will show a slight falling off in rapidity of action, whilst the fourth will most likely exhibit traces of thinness, with an increased slowness of action in the developer. The iron developer is practically the only one which admits of being revivified, and the best way to effect this, in the writer's experience, is to make, for half a dozen plates, half as much developer again as the quantities given above, and after each development to pour the used developer back into the fresh. By this means the loss of power is much more evenly distributed over the whole number of plates. The subsequent processes are the same with iron developer as with the others, except that it is advisable to add acetic acid, in the proportion of one drachm to each pint, to the water used for washing before fixing, in order thoroughly to remove the iron. After this, the plate should be well washed with plain water before fixing, as if put into the hypo. bath with the acid upon it, a very serious loss of density will ensue.

The above paragraphs contain as much as can probably be usefully said with reference to the development of lantern slides, experience alone can ensure success, but the beauty of the results is so great that the trouble and expense involved in attaining them are more than compensated for.

The habits of care inculcated by bromide paper printing are of the greatest value in lantern-slide making; the treatment required by any particular negative is much the same in both cases, and the cost of the paper being about half that of the plates, the former may be recommended for practice. With the exception of the pyro-developer, all those enumerated above as suitable for lantern-slides will give excellent results, of the same character, with bromide paper. The pyro-developer gives disagreeable rusty-brown tones, and cannot be recommended for bromide paper work. The treatment to be given to the paper is in all cases the same as that required by the slides, except that the acid clearing solution should not contain iron, but should be a saturated solution of alum with one drachm of hydrochloric acid to each pint of alum solution.

It only remains to indicate the steps to be taken in order to complete the lantern-slide. After the final washing the plate is to be gently brushed with a soft camel-hair brush, under the tap, to remove any particles adhering to the film. It is then dried in a draining rack, without heat. If more than one plate is to be dried at once, sufficient room for circulation of air must be allowed between them.

The materials required for completion are a supply of gummed strips of black paper for binding, a sufficient number of thin, clear glasses of the same size as lantern slides, and a box of gummed "spot" labels. If it is desired to cut off a portion of the field, a supply of masks of various shapes and sizes will be required, and these may be purchased, or cut out of stout black paper, laid on glass, with a sharp penknife or "print-trimmer." If the slides are to be labelled, either the required details may be written upon a white label, affixed to the slide, or masks may be obtained which are white upon one side (this side being in that case put next to the cover-glass), or the inscription may be written in "Chinese white," ground in water, upon the black mask.

The dry slide is taken, brushed with a camel-hair "softener," the mask, if to be used, adjusted upon it, and a thoroughly cleaned cover, free from scratches, laid on the top. A gummed strip is now moistened, preferably with very thin flour-paste, and laid flat upon the table. The combination of slide, mask, and cover is raised, pressed firmly together, and placed vertically upon the strip, one corner of the slide corresponding with one end of the strip, and occupying its centre. Slide and strip are rotated together so that the next following side may, in its turn, become attached to the strip, and so on with the two remaining ones. The superfluous portion of strip is next cut off, and the two loose ends drawn tightly down on each side of the slide and secured there. At each corner a V-shaped portion is cut off on both sides with a pair of scissors held at a slight angle to the glass, and the sides are then successively drawn tight over the edge, and rubbed down into close contact with the slide. It will probably be found at first that some portions will not adhere ; these must be remoistened and rubbed down, and the finished slide is then allowed to dry, and subsequently cleaned by wiping the glass with a damp (not *wet*) cloth. Dirty slides in the lantern are an abomination.

Finally, the slide is marked. This is done by holding it up so that the picture is in its proper position, and putting one of the spot labels at *each* of the *top* corners. When the slide is put into the lantern these two spots are to be at the bottom, on the side nearest the light, and the picture will then appear upon the screen in the right position.

## CHAPTER XV.

### COPYING.—ENLARGEMENT.—REDUCTION.—PHOTOGRAPHY OF CULTURES OF BACTERIA.

Photographic lens—Copying—Enlarging—Reducing—Illumination of negative — Production of a second negative — *Photographing cultures* — Detail obtainable—Tube cultures.—Stab and stroke—Illumination—Use of tank —Agar cultures—Plate cultures.

THE method of making lantern slides by contact, with subsequent development, is the only one treated of above. Better results may in some cases, especially with thin negatives, be secured by the use of the copying camera, but both this and the enlargement or reduction of a negative for the production of a lantern slide, involve the use of a photographic lens of the ordinary character.

The possession of such a lens is a very considerable advantage, and the writer has obtained excellent results by the use of an ordinary half-plate lens. A smaller one is not advisable, since, unless it be of very excellent quality, its "covering-power" will not be sufficient to give a sharp image right up to the edge of a lantern slide. In the writer's copying apparatus he has the lens fitted to a slide which takes the place of the one which carries the connecting tube between the microscope and camera, and he thus utilises his photomicrographic camera for copying purposes.

The negative is supported in a frame at a suitable distance in front of the lens. When the negative is simply to be copied, the negative and the plate will be equidistant from the *diaphragm* of the lens. When the negative is to be reduced, it will be found necessary to place it farther from the lens, and to close up the camera to a corresponding extent. When enlargement is required, the negative must be nearer to the lens than the plate is.

The negative must, of course, be illuminated, and evenly too, by transmitted light. This may be effected by placing a sheet of white paper behind the negative, and allowing the light to fall on it, under as small an angle with the camera line as possible, the lamp being carefully shaded on the side next to the camera. Or, the whole apparatus may be placed opposite a window, outside which a white screen is placed at an angle of 45°, so as to reflect the light of the sky through the negative. Or, the negative may be illuminated by a condenser, placed closed behind it, and of a diameter greater than its own, in the principal focus of which a source of artificial light is placed, so that the negative is illuminated by a bundle of parallel rays of great intensity. In this case the light must be carefully adjusted so that its rays are evenly distributed by the condenser. In any of these proceedings the greatest care must be taken to secure absolute sharpness upon the screen, by using the ground glass first, and then the ruled clear-glass screen with the Steinheil lens.

Copies, enlargements, or reductions, may in the same way be obtained upon bromide paper. The latter is placed in a specially designed carrier, manufactured by the Eastman Company, and is exposed in the same way as the lantern plate would be. The processes of development and completion are precisely those previously described.

If several copies of a size different from that of the negative are required, it will be found best to make a transparency first, and from this a second negative, either by contact- or camera-exposure, from which any number may be printed. In this case the need for extreme sharpness in the first reproduction is even more absolute than in the former case.

In connection with this question of work to be done with the ordinary photographic lens, we proceed to describe the methods to be adopted for the representation of some objects which, whilst not coming strictly within the domain of the photo-micrographer, are yet such as the practical worker may often find himself called upon to deal with. More especially is this the case when the camera is called in to aid the work of the investigator.

The bacteriologist who desires to represent the appearances of his cultures, whether plate or tube, or the biologist who wishes to reproduce in their entirety large sections, or series of sections, for the purpose of obtaining general views, will find the resources of ordinary photo-micrography inadequate, and the photographic lens must be made to supply their place. The process then becomes perfectly simple.

For such purposes as those dealt with in the earlier portion of this chapter, and those to be now adverted to, it is not necessary that the lenses employed should be of very wide angle or great rapidity of action. As already said, the writer uses a half-plate lens, and one of a very inferior character from a technical point of view, but which nevertheless answers his purpose extremely well; it has a flat field, good covering power, and correct coincidence of visual and actinic foci, and he has found it equal to his requirements for copying, enlarging, reduction, and the photography of cultures.

As regards the reproduction of opaque objects, the process, except as regards the methods of lighting, is precisely the same as in making lantern slides under the same conditions of size-relation between object and image. The lighting must of course, for opaque objects, be so arranged that they are illuminated from the front in such a way as to bring out the required details. As regards detail one remarkable fact has to be stated. In photographing echini, with the spines upon them, at one-third the natural size, the writer has found that the extremely delicate markings visible upon the spines when closely examined by the naked eye are as well defined in the reduced photograph as in the original object, and far more strikingly apparent. With an object of such depth as an echinus, and, indeed, in all the processes referred to in this chapter, it is very important to use as small a portion of the aperture of the lens as possible—in other words, to use the smallest stop which will give good definition, in order that the focus may be sufficiently deep. This applies with especial force to cases in which the object is to be taken under a slight magnification; with simple copying a larger aperture is allowable.

When transparent objects have to be dealt with, the lighting must necessarily be arranged so as best to counterbalance the difficulty presented by want of contrast, or any other cause, on the lines already laid down.

As an example of this class we select tube cultivations in gelatine. There is no difficulty whatever in showing up the culture, since by simply placing the tube against a black background the actual growth can be made to show up against it with startling distinctness. To show the tube, however, at the same time with the culture, is a very different matter, and the writer tried many plans before he hit upon the one which he now adopts, and has used with, in most cases, very satisfactory results.

The method of treatment must be varied according to (1) the nature of the culture medium, as regards outline and transparency ; and (2) the character of the growth upon it.

Let us take first the case of a "stab" cultivation in gelatine. The conditions here will be a thin-walled tube, containing a cylindrical mass of transparent gelatine, with a central growth. The essential sources of difficulty are, the thin-walled tube, offering practically no contrast, and the gelatine, which, owing to its refractive index and its cylindrical (and therefore) lenticular form, will, if the growth be so extensive as to come nearly or quite into contact with the tube, prevent the margin of it from being rendered. To indicate the source of difficulty is to suggest the method of removing it. The writer immerses the tube in a parallel-sided glass tank of boiled and filtered water, and places this in front of the lens. The illumination is obtained by placing behind the tube, and considerably above its level (at least 12 inches), a lamp the rays of which are so concentrated by a bull's-eye condenser as to throw a flat bundle of more or less parallel rays through the tube so obliquely that they do not enter the lens itself, which only receives those reflected by the tube and the cultivation. The requisite amount of contrast in the background is obtained by placing a sheet of white paper, resting against the support of lamp A, at a distance of about 18 inches behind the tank. By the side of the tank, *and as close to it as possible,* is placed a second lamp, B, which illuminates the paper, but not the culture, and the image is now carefully scrutinised upon the ground glass, and the relative intensities of A and B adjusted to produce the best effect with the particular growth which it is wished to reproduce. Both lamps must be so placed, or so screened, that no direct light from them enters the lens. The object of the second lamp is to secure a certain amount of transparency in the ground, which would otherwise be quite black and opaque before the culture is sufficiently printed (speaking, that is, of the print, and not of the negative). By thus lightening the ground, the outline of the tube, as well as of the culture, may be satisfactorily displayed, even in the case of growths so delicate as those of "swine erysipelas" or "mouse septicæmia."

In the case of "stroke" cultivations the method will require to be varied. The lenticular form of the culture medium is less obtrusive, but will depend very much upon the extent of the obliquity. The more nearly parallel to the long axis of the tube the culture medium, the less will be the difficulties from its form, and from the fact that it is situated obliquely to the axis of the tube.* It will generally be found best, in dealing with stroke cultures, to dispense with the tank, unless the growth be so wide as to approach the edges of the tube.

In any case, the greatest pains must be taken to secure even illumination of the whole extent of the

* For this reason the writer always slopes his culture media as much as possible.

growth, or the results will be unsatisfactory. In dealing with agar cultures this is especially necessary. This medium is, of course, less transparent at the lower portion of the tube; more light must therefore be concentrated upon the lower portion of the growth than upon the upper.

With plate cultivations the best results will probably be secured by removing the lid of the plate, and (if it is to be preserved) replacing it by a sterilised glass plate. The whole is then placed upon a background of black velvet, and illuminated by a lamp on each side, the camera and lens being placed vertically over it. In the writer's apparatus both camera and lens-carrier can be clamped to the board, and this last turned up, so as to give the camera a vertical position.

In some cases, especially where no liquefaction has taken place, the plate may be supported vertically in front of the camera, and the light sent through it obliquely from the back. This plan answers especially *well* when the growth is very delicate and filmy and is confined to the surface. It succeeds *worst* of all when there is growth both in the depth and in the surface, since the shadows of the colonies in the former position are apt to be cast very distinctly upon those in the latter, and the colonies themselves being also visible the impression produced is a misleading one.

To produce the best results, the glass plates themselves should be clear and even. Those with "puntied" bottoms are very unsatisfactory.

Reduction, enlargement, or simple reproduction as to size, will, of course, depend upon the relative distances of plate, lens, and object.

The focussing should in every case be completed upon the ruled clear screen, with the aid of a Steinheil or other similar lens. The conditions of illumination and contrast, as well as sharpness, can in this way be better judged of than even upon ground-glass, however fine.

It will be observed that the above directions are based upon precisely the same principle as the oblique (or dark field) illumination of microscopic objects, and in some extremely trying cases the writer has carried the similarity so far as to arrange an upright rod, of suitable width, between the tube and the source of light. A very striking example of the results will be found in the Report of the Medical Officer of the Local Government Board for 1890–91,* where not only the diphtheria colonies, but the delicate outline of a drop of milk which had been allowed to run down the surface of the gelatine, are very distinctly reproduced.

* Plate III. Fig. 10. Plates III., IV., VIII. and IX. contain numerous examples of the writer's photographs of tube-cultures, and still more difficult ones will be found in the forthcoming report on the influenza epidemic.

# CHAPTER XVI.

## THE FRONTISPIECE.—CONCLUDING REMARKS.

The interest of a sermon lies, or should lie, in its application, and upon the same principle it seems desirable to illustrate, by means of the frontispiece, some of the most important points which have been dealt with in previous pages.

All the photographs reproduced have been taken specially for the purpose, and this being kept in view, Edwards' orthochromatic films, of medium rapidity, have been used in every case, and development by hydroquinone has been the order of the day. The experience accumulated in the work has been very considerable, and it is proposed to present it here in a condensed form.

In selecting an object for the 70 mm. objective it seemed desirable to choose an insect mount, and having a very beautiful specimen of *Nycteribia* (a parasite of the flying fox) in his possession the writer began upon that. Upon the first joint of the abdomen of this insect is a girdle, made up of a number of closely set, dark-brown bars, with narrow spaces between them, but as the spaces were no darker than the general ground of the body, there seemed no reason to anticipate any difficulty in the reproduction, and it was undertaken with a considerable amount of confidence. Negative after negative was made, with all kinds of variations of screen, light, and exposure. At first the results improved, up to a certain point; after that they remained stationary. The spaces between the bars of the girdle obstinately refused to show up as strongly as the equally dark body, and when they were fairly visible the legs, hairs, and, in fact, almost every other detail, was "not lost, but gone, before"—the spaces began to develop. Plenty of *passable* results were obtained, but as the writer has always steadily excluded "that will do" from his photo-micrographic phrase-book, he persisted until it became evident that there was some optical law antagonistic to his success. He believes that the failure was due to the fact that since the only rays transmitted by the spaces are those of low refrangibility, he was in fact photographing a diffraction-grating, the interference of which with the rays required, caused the dispersion of most of the rays entering from below; and this still more from the fact that as the rays entered obliquely from the bull's-eye, the spaces in the grating were thereby still further contracted. At any rate, it was not possible to produce a negative which could be relied upon to give a good result in collotype; a further effort, at a far higher magnification—*i.e.*, with a much longer camera, may, as is the case with diatom work, yield a more satisfactory result, since the increased magnification involves a much closer approximation of the objective to the object, with a consequent virtual increase of angle in the former which may allow the more oblique rays to be picked up. At the present moment the necessity for going to press does not permit the experiment to be tried. It should be added that heavy development with subsequent reduction, and light development with Rodinal, alike failed materially to improve the result.

As regards the frontispiece itself, the first photograph is that of the temporary tooth in a kitten's jaw, with the germ of the permanent one, prepared with chromic acid, softened with hydrochloric acid, and cut in wax and oil in a hand microtome of the author's design, being subsequently stained with carmine. The section itself is an extremely perfect one, not remarkably thin, but bisecting the pulp cavities of both tooth and germ, a very unusual circumstance.

The stain in this case was taken up most heavily by the tooth and the bone of the jaw, both being dark crimson; whilst the pulp, and also the gelatinous tissue surrounding the germ, were hardly stained at all in comparison. It was therefore necessary to use a fairly dark screen (yellow, of course), and to over-expose somewhat, the development found most successful being Rodinal. A large bull's-eye was the condenser.

As to the second photograph, the spine of *Echinometra lucunter*, it is not necessary to say more than that it was taken strictly upon the lines laid down on p. 105.

The third photograph was taken from a section of the cat's whisker, in its follicle, with the blood-vessels injected, made by the writer many years ago. The material was prepared with spirit, infiltrated with gum, and cut in the same manner as the tooth, the stains being picro-carmine and logwood. The outer root-sheath is here divided into two parts by a large annular sinus, and only its inner layer is represented, whilst Huxley's and Henle's layers are amalgamated.

A pale yellow screen, and a moderate exposure were given, the objective used being an apochromatic 16 mm. with No. 3 projection-ocular; the Abbe condenser, with the front, and a small bull's-eye, formed the illuminating apparatus.

For the fourth photograph the writer had intended to use the optical combination just mentioned, but the diatom, *Arachnoidiscus Ehrenbergii*, proved to be just too large for the flat part of the field of the objective. The writer therefore tried the semi-apochromatic 8 mm. of Reichert, the result being as shown. The Abbe condenser was in this case used without the front lens, a bull's-eye being also employed.*

The fifth figure shows the thrush fungus, *Saccharomyces mycoderma*, cultivated by the writer from the mouth of a patient, the medium in this particular case being acid beef-broth, with the addition of cane sugar, and the preparation being made after thirty hours' growth. The stain was methyl-violet, and the objective was the apochromatic 4 mm. with No. 3 projection-ocular, and the full angle of the Abbe condenser, the magnification being 500 diameters. No bull's-eye nor screen was used.

The sixth photograph is that of the *Spirillum Choleræ Asiaticæ*, magnified 1000 diameters, the preparation having been made by the writer from a culture ten hours old, and the stain being Loeffler's ferro-tannate solution followed by methyl-violet. The process is an uncertain one, but the preparation in question happens to be extremely good, though the mordant has to some extent contaminated the ground. It was taken with the apochromatic 2 mm. of N. A.

* When the bull's-eye is used the radiant must be placed at about a foot from the condenser, or the results described cannot be obtained.

1.3, and the full angle of the Abbe condenser. The roundness of field in the apochromatics is shown at the edge of the photograph. No screen nor bull's-eye was used.

The explanation of the absence of the screen is that in this, as in all the other cases, lamp-light, from a paraffin lamp with a three-quarter-inch wick, was the radiant employed; the writer's oxygen-cylinder having been exhausted in his experiments on the parasite, he turned to the next illuminant at hand, and found his previous views amply corroborated. The flame was used on edge in all cases but the first.

It only remains to be said that none of the objects were prepared specially for photography.

In view of the remarks made in the present chapter, the writer may perhaps be forgiven for saying that the secrets of photo-micrographic success are two.

The first is the close study of failures, so as to elucidate (and eliminate where possible) their causes.

The second is a capacity for taking pains; the idle man, the man who objects to worry over minutiæ, the man who jumps to conclusions, the man who is satisfied with inferior results, will never attain to success in this department of science; whilst, on the other hand, close application, unsparing self-criticism, and emulation of the best results of other workers, will lead on to successes which at first seemed beyond attainment, and will justify the motto with which, as in the previous edition, the writer lays down his pen:

LABOR OMNIA VINCIT.

# APPENDIX.

# APPENDIX.

## PREPARATION OF SECTIONS FOR PHOTO-MICROGRAPHY.

Fixing agents—Dehydration—Staining in mass—Infiltration with paraffin—Imbedding — Microtomes — Cathcart — Minot—Caldwell—Celloidin work—Mounting sections — Clearing — Staining reagents — Decolorisation — Dehydration — Finishing — Double staining — Objections to it — The writer's modification of Weigert's stain.

THE preparation of objects for photographic purposes is a matter of so much importance that I propose to give a short summary of the methods which have in my hands proved most successful.

Wherever possible, and there are comparatively few exceptions, the number of which is constantly lessening as methods are improved, the paraffin process is the one which I adopt for cutting.

Briefly, it is as follows:—

The objects, if preserved in Müller's solution,* or other chromic compound, are washed in running water for 24 hours, or until the water ceases to take up the yellow colour when allowed to stand for a short time. They are then placed in alcohols of gradually increasing strength† until they reach 70 % alcohol. They are then, if to be stained in the mass, which should be done wherever it is possible, placed in Grenacher's alcoholic borax carmine or Kleinenberg's alcoholic logwood, both of these being extremely good for photographic

* 2 % of bichromate of potassium with 1 % of sulphate of soda.

† The first may consist of one part of methylated spirit to two of water, the next of equal parts of spirit and water, the third of two parts of the spirit to one of water, and the fourth of four parts of spirit to one of water, which is almost precisely the classical 70 % alcohol.

purposes. If the carmine stain be chosen, the next step will be to place the stained object in 70 % alcohol containing ¼ % of hydrochloric acid, which renders the diffuse stain of the carmine solution a most beautifully precise one. The acid alcohol should be allowed to act until the object appears (not becomes) transparent. The difference is really in the character of the colour and not in translucency at all, but is easily recognised as it appears. From this point, whether Grenacher's or Kleinenberg's stain be used, the process is the same. Pure methylated spirit is next used, and finally absolute alcohol to complete the dehydration. The object should be placed in a small quantity, changed about twice, rather than allowed to lie at the bottom of a larger quantity.

A tube is now taken, of sufficient width to hold the object, and about six or eight times the depth of the latter. The bottom third of the tube is to be filled with chloroform or oil of cedar wood, and then absolute alcohol very carefully poured down the side of the tube, so as to lie upon the other fluid with only very slight mixture at the point of junction, and to about the same depth. The object is now rapidly but gently transferred to the tube, the latter is then corked and allowed to remain at perfect rest until the tissue has sunk to the bottom. With chloroform this will happen very slowly, or not at all, so that it is recommended to add to the chloroform as much ether as will bring its specific gravity to about that of water or a little over. With oil of cedar-wood this trouble never occurs.

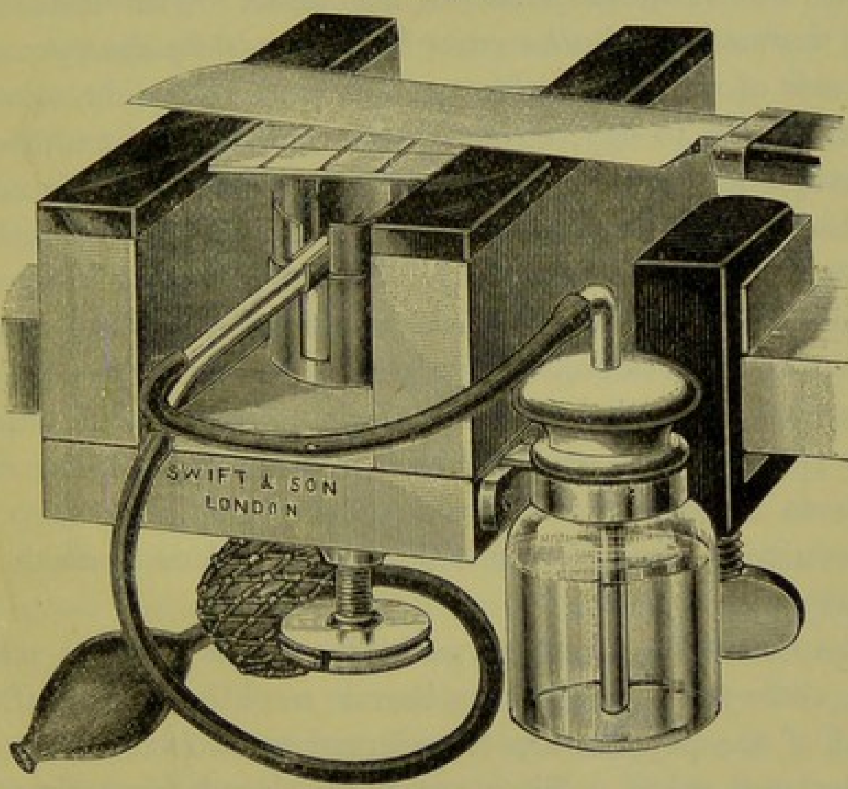
The next process is to penetrate the object with paraffin. The object is rapidly removed from the tube containing two layers of fluid to one containing only the lower fluid. If the latter be oil of cedar, the object may be then at once transferred, after draining on filter paper, to a bath of paraffin having a melting point of from 42° to 52° Centigrade.* In

* The exact melting point to be chosen is a matter of controversy. The best plan is to keep objects in methylated spirit of 70 % alcoholic strength, and to dehydrate when wanted for use, regulating the melting point of the paraffin by the temperature of the laboratory. At 5° C. paraffin of 42° will just "chain," at 8° it will do so well, at 15° the paraffin should have a melting point of 48°, and at 20° paraffin of 52° is none too hard, beyond this temperature tissues are apt to suffer seriously. Objects may, however, be kept in cedar oil or chloroform for any length of time without deterioration in the dark.

this bath the objects remain until they have become quite opaque again, and are then transferred to a second one, in which they remain until the odour of the oil has passed quite away.

With chloroform the process is somewhat different. The objects are placed in a tube with enough of the chloroform (or chloroform and ether) to rather more than cover them, and small pieces of the paraffin selected are added, first to make a saturated solution in the cold and then with warmth and gentle agitation till the tube is full. It is then allowed to stand for a short time, and finally the object is transferred to

Fig. 34.




a melted bath of the same paraffin, where it remains until the smell of chloroform has disappeared. Imbedding is then accomplished, either in a watch-glass or in a box of paper or capsule metal, or, best of all perhaps, in a box made of two L-shaped pieces of brass, placed upon a glass slide, and the whole is then cooled as rapidly as possible.

The sections are now to be cut, and this, constituting as it does the essential advantage of the paraffin process, is a matter of the greatest moment. Some form of microtome must be used. The ordinary "Cathcart" (Fig. 34), with a plane iron, will do excellent service, with care, where single sections are required. For serial work, and especially for photo-modelling, a process of the greatest value in biological work, the finest microtome known to me is the "Minot," and having worked with microtomes of nearly all, if not all, types, I unhesitatingly place it first, although the automatic ribbon action of the large Caldwell microtome gives it the advantage in this one particular, that when all is in order there is absolutely nothing to be done but to turn the handle until the ribbon is filled.*

The next step is the placing of the cut sections on the slide. If single, they are lifted up with a camel-hair pencil, laid on a clean slide, and sufficient filtered water run in under them from a pipette to lift them quite off the slide. The slide is then gently warmed *until all creases have completely disappeared*, and the excess of water is then run off and the slide allowed to drain and then to dry, either spontaneously or at a temperature well below the melting point of the paraffin. If cedar oil have been used in impregnating with paraffin, methylated spirit or a weaker alcohol may be used instead of the water; if thoroughly impregnated with paraffin this is unnecessary.

The next process consists in placing the *thoroughly* dry slide in a wide-mouthed bottle of clean mineral naphtha, which rapidly removes the paraffin, and if stained in mass nothing remains to be done but to drain off the naphtha, drop xylol-balsam on to the section or sections and place a clean cover-glass over all.

If the tissue has been cut unstained, the sections adhering to the slide are placed in mineral naphtha,† and after the removal of the paraffin are transferred to two successive bottles of methylated spirit. They are then placed (face downwards if possible) in a dish, and the staining fluid selected is so added as to fill up the whole space below without bubbles. The time required for staining will vary according to (1) the nature of the object; (2) the method of preparation; and (3) the dye selected. For further information upon these pcints the reader

* This microtome is no longer on the market.

† Wherever mineral naphtha is mentioned it may be understood that ordinary petroleum, such as is burnt in lamps, will answer equally well, and is only about one-twelfth of the price, costing as a rule about sevenpence per gallon.

must be referred to works treating of histological or bacteriological technique.

The decolorisation is effected by immersing the slides in two successive bottles of absolute alcohol, the first being used until all excess of colour has disappeared, the second to cleanse the slide, and complete the dehydration. The staining, clearing and dehydrating having been effected, the slide is placed in two successive bottles of mineral naphtha, to remove the last traces of absolute alcohol, and is then allowed to drain, but not to dry, and after the application of sufficient xylol-balsam, the cover-glass is placed upon the preparation.* The above process may appear, and indeed is, somewhat lengthy and complicated, but the results it yields, from both the scientific and the photographic points of view, are unapproached by any other process. The method of celloidin imbedding was much practised some time ago, but it has two great disadvantages. (1) Thin sections are very difficult to cut, in consequence of the elasticity of the medium, and even ones, without ridges or cross markings, are still more troublesome to obtain. (2) The medium takes the stain, and does not yield it completely up again under any ordinary method of treatment, so that the interspaces of the tissue are filled with a coloured ground, and delicate structural details are very much obscured.

The old wax and oil method, in which a block of tissue was simply imbedded and cut without being impregnated, has yielded good sections, but if thin enough for photography they generally fall to pieces in staining. After nearly twenty years' constant experience, I have settled down steadily to paraffin impregnation, and cut every tissue by this process. It gives perfectly flat sections, of even thickness, and this no other process does; whilst the great freedom in handling conferred by the fact that the sections are firmly adherent to the slide during all the staining and mounting processes, is a factor of inestimable value. That it requires time is a small objection in comparison with the great advantages obtained, and the fact that structures can be so readily followed through a whole series

* I have long given up the use of essential oils after dehydration. However high the refractive index of those used may be, the ultimate index is that of the xylol-balsam used for mounting. Naphtha has also the advantage that it is entirely without action upon aniline dyes.

of sections and their true form made out with almost mathematical accuracy.

For tissues too hard to be cut in a sliding microtome, a plane-iron and Cathcart microtome must be used, and with care may be made to yield ribbons of sections of very fairly even thickness.

The question of staining is one of the greatest importance for photo-micrography, and it will almost invariably be found that those stains which yield the best negatives also give the most perfect preparations for purely histological purposes. Four stains stand out from all the others as being the best for both purposes. They are hæmatoxylin, Grenacher's alcoholic borax-carmine, methyl violet, and safranin. The first for general histological and pathological work, the second for that purpose and for zoological work, the third for bacteria, and the last especially for cytological work, though also for general purposes, are most strongly to be recommended. They are all *precise* stains, and upon sharp staining depends to a very large extent the perfection of the image.* Methyl blue should, as far as possible, be avoided. It is not a precise stain, and is very easily washed out, so that the preparations are apt to be very faintly stained, and of all photographic abominations there is *nothing* equal to a pale methyl-blue-stained preparation.

It is a rule with me never to double-stain where it can possibly be avoided. If sections be sufficiently thin (they cannot be too thin for this purpose) and as deeply stained as possible, nothing is gained, either for study or reproduction, by mixing colours which, after all, as a rule, only look pretty. There are a few cases, no doubt, in which it is indispensable, but they are very few indeed, and selective staining is a very poor method of making good the defects of a thick section.

For staining cover-glass preparations of bacteria a very good stain is made by adding two drops of a saturated solution of methyl violet in absolute alcohol to half a watchglassful of anilin water, and then adding sufficient methylated spirit to

* For methods of preparing and using them the reader is referred to Bolles Lee's "Microtomist's Vade-Mecum," issued by the publishers of the present work.

dissolve the iridescent pellicle upon the fluid. Preparations are very rapidly stained, and, as a rule, only require thoroughly washing out in distilled water. If this be not sufficient to clear them, a little more spirit should be added to the dye for the next preparation. If they are not stained sufficiently, the time allowed for staining must be increased. In the case of pus from gonorrhœa, about five seconds will be amply sufficient to stain sharply both the pus cells and the organisms, and the resulting preparations are admirably adapted for photo-micrography.

# INDEX.

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