First steps in photo-micrography / [F. Martin Duncan].

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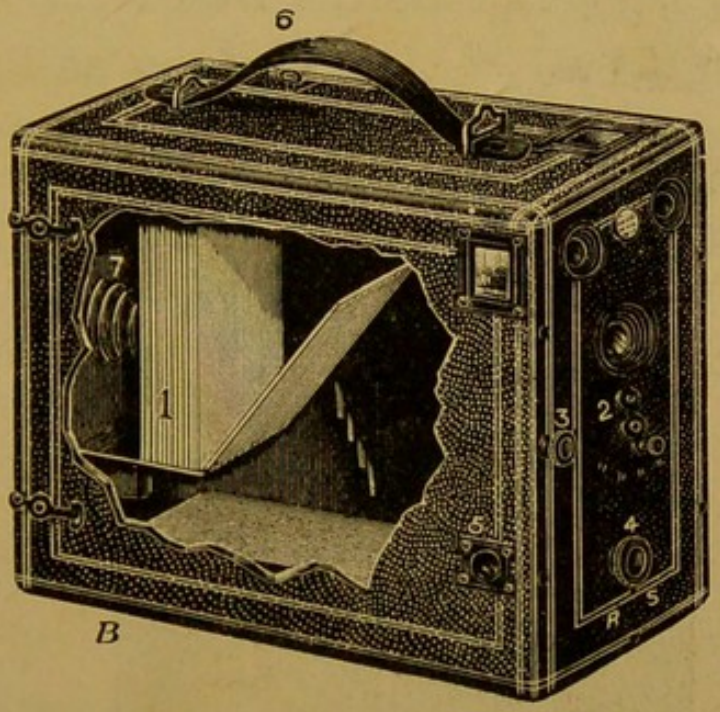

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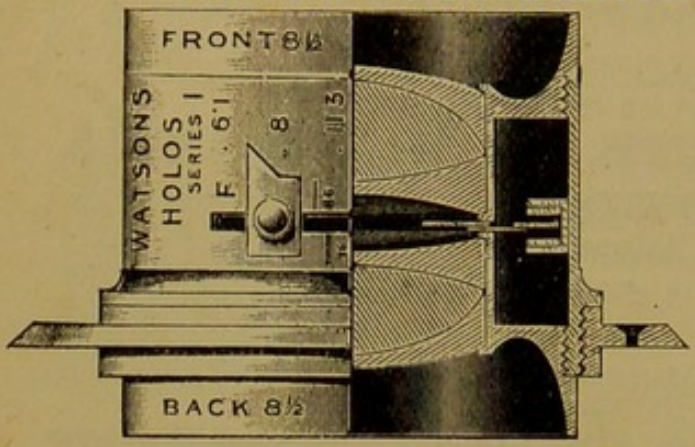

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"The largest effective aperture possible was one inch in diameter, so it will be seen that the lens was capable of working at f/6·4. *With this stop it could be got to cover a whole-plate easily with good definition, and on a ten by eight plate, only the extreme corners were unilluminated, the angle of the cone of rays being* 86°. *It will be observed that the actual angle required by a half-plate lens of this focus is* 64°, *so that there was an excellent margin for the use of a rising front, whether the plate were used vertically or horizontally.*"

"The tests applied for achromatism showed conclusively that the correction in this respect left nothing to be desired, while in the matter of distortion, the image of a straight line the full length of a half-plate, falling right upon the margin of the plate, did not vary from the straight more than a hundredth of an inch in its whole course—an amount quite imperceptible even to a practised eye without instrumental aid. The curvature of the field was similarly infinitesimal. Tested on a flat subject, ON A WHOLE PLATE, the extreme edges were at their sharpest focus, with full aperture, only one-fiftieth of an inch nearer to the lens than the centre. This was the mean of a series of readings, which did not vary amongst themselves a hundredth of an inch altogether, and should suffice to show that the lens is not merely a medium angle high rapidity lens, but should prove a very valuable wide-angle instrument also."

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FIRST STEPS IN PHOTO-MICROGRAPHY

A HAND-BOOK FOR NOVICES

BY

F. MARTIN DUNCAN, F.R.H.S.

WITH ILLUSTRATIONS

[THE AMATEUR PHOTOGRAPHER'S LIBRARY, No. 23.]

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PREFACE.

In writing this little book, I have endeavoured to supply a hand-book essentially for the beginner—namely, a simple and, as far as possible, a non-technical account of the methods and apparatus employed in the production of Photo-micrographs. For this reason, I have refrained from going into any details likely to bewilder or alarm the tyro, and have devoted the space at my command to a description of the manner in which he may most successfully surmount those difficulties which are sure to beset his first attempts. For, given a certain modicum of success from the very first effort, the interest is bound to increase, so that what was perhaps begun as a casual form of

amusement, eventually becomes an absorbing and life-long hobby.

Photo-micrography has made such great progress towards perfection during the last few years, that it is now rapidly becoming a very popular hobby. To the bacteriologist, medical student, botanist, and to all who are interested in natural science, it is of the greatest importance, enabling them to keep a faithful and accurate record of their observations and experiments.

If the instructions given in these pages are carefully followed, the novice should have no difficulty in gaining an insight into the mysteries of this most fascinating branch of Photography. When he has successfully mastered these first steps and gained some amount of experience and facility, he may then, with advantage, consult the more exhaustive and technical works.

F. MARTIN DUNCAN.

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Part I.

CHAPTER I.

LOW-POWER PHOTO-MICROGRAPHY.

It is surprising how very few amateur photographers possessed of a good stand-camera, and fairly short-focus lens, realise that the pleasures and interests of low-power photo-micrography lie within their reach. This is no doubt largely due to the popular, but quite erroneous, idea that photo-micrographs cannot be produced without the aid of a microscope. It is a great pity that this idea is so general, for it has prevented many from taking up a most interesting branch of photography, and one which only requires to be better known to become very popular.

Literally, photo-micrography means photographing a magnified image. How the magnification is obtained, depends entirely upon the subject, and the number

of diameters it is to be enlarged. If it is the infinitely small that we wish to photograph, such as the finest markings on a diatom, or a group of bacteria, objects which require a magnification of at least one thousand diameters, then a microscope and powerful battery of lenses must necessarily be employed. But supposing we wish to obtain a photograph of one of the numberless beautiful and interesting objects which are sufficiently diminutive in size to be rarely noticed, such as a baby spider, or wee, dainty flower, which, because of its small size, is

> "Born to blush unseen,
> And waste its sweetness on the desert air,"—

then the required magnification can be much better obtained with a short-focus lens, or very low-power micro-objective attached to the camera, than with a microscope. Indeed, many of the countless objects which lend themselves to low-power photo-micrography, are, from a microscopical point of view, comparatively large, as they cover a wider area than the

lenses and body-tube of the microscope are constructed to show; and do not require a higher magnification than from one to twelve or fourteen diameters, to show them to perfection.

Another mistaken idea, which has helped to keep many people from taking up photo-micrography as a hobby, is that a very expensive outfit is required. Now, as a matter of fact, this is not at all the case, as I hope to clearly prove in the following pages. The necessary apparatus can be obtained for a very modest sum, and this amount can be still further reduced by any one with but an elementary knowledge of carpentry.

The apparatus required for low-power photo-micrography is a good, solidly built stand-camera, capable of a bellows extension of at least twelve or fourteen inches (sixteen to twenty inches would be very advantageous), one or two lenses of varying focal length, and some form of stand to carry the camera, and the object to be photographed. For both economical and manipulative reasons, a good quarter-

plate stand-camera will be found the most useful size to work. It should be as solid as possible, should have a reversing back, so that both oblong and upright pictures can be taken; and for low-power work, may with advantage be fitted with a rising front, though this is not an absolute necessity, and is almost better dispensed with altogether, if the same camera is to be used for medium and high-power photo-micrography.

The stand or baseboard to carry the camera and object to be photographed, can be easily constructed at home. All that is required is a truly planed one-inch pine or deal board, about five feet long, and ten inches broad, with a perfectly straight and central line drawn down one side to act as a guide for centring the camera, etc. Two solid blocks of wood, four inches high, two-and-a-half inches broad, and from six to eight inches long (according to the length of the base of the camera), are screwed on to one end of the baseboard, to form a stage to which the camera can be firmly fastened. Two rails,

FLUSTRA FOLIACEA, × 2.

preferably V-shaped, on which the object-holder will travel, run from the camera stage down the entire length of the board.

On to a piece of board, ten inches long and four inches broad, two pieces of wood, with grooves corresponding in angle to the rails on the base board, must be securely fastened; this will form the movable base of the object-holder. A good stout frame, measuring ten by eight inches, and two inches deep, is next made; the top piece being hinged, and made to fasten on one side with some form of hook-and-eye. To produce a very useful cross-movement, two fillets of wood are fastened on to the top of the movable base, so as to make a groove into which the frame can be pushed; while a rising and falling adjustment will be obtained by screwing narrow fillets of wood on the inside of the frame, to form a central groove on each side, for the light boards which act as object-carriers, to slide into; the carriers being fixed at the desired height by means of thumbscrew clamps.

One of the carriers must have an accur-

ately central hole about two inches in diameter, below which must be inserted a pair of spring clips, similar to those used on the microscope stage for holding the object in position.

The choice of lenses depends upon the amount of magnification required. If the objects are comparatively large, such as a rose beetle, or orange-tip butterfly, a magnification of two or four diameters will be sufficient; and for this work a portrait lens of about three-inch focus will prove to be very useful. For objects requiring a magnification of seven or eight diameters, very fine results can be obtained with a small rectilinear lens of about one-and-three-quarter-inch focus, specially made for low-power photo-micrography by Messrs Dallmeyer. The "Planars" of Zeiss, and Zeiss-Ross, are beautiful lenses for this work, but are rather costly.

Provided the object to be photographed requires a magnification *exceeding* ten or twelve diameters, a good three-inch or two-inch micro-objective, which may often be purchased second-hand for a very

moderate sum, can be used. As they are specially made for use with the microscope, it should be borne in mind that they are corrected for a comparatively small field of view; but if mounted on the front of the camera by means of a flange, in a similar manner to the ordinary photographic lens, they will be found exceedingly useful for photographing small objects requiring a magnification of between twelve and sixteen diameters, and therefore only a comparatively restricted field of view. If, however, a photograph of such a comparatively large object as a wasp or full-grown garden spider be attempted with one of these microscopic objectives, only the central portion of the picture will be found to be in focus, while the margins will be hopelessly "fuzzy-wuzzy."

For objects to be photographed by reflected light, "daylight" may with advantage be used as the source of illumination, particularly if a black or dark-coloured background can be employed.

To take a photograph by reflected light,

the low-power photo-micrographic stand, with camera and object-holder attached, should be placed close to a well-lighted window having a north aspect, and arranged in such a position that the object to be photographed is evenly lighted. The specimen to be photographed, is fastened on to the object-holder in such a position as to display to the best advantage that portion of which it is desired to obtain a photograph; and when this has been satisfactorily accomplished, both object-holder and camera must be moved nearer or farther from each other, until the desired magnification is obtained. Only the very finest grained ground-glass should be used as a focussing screen, otherwise it will be impossible to focus sharply the finer lines and more delicate markings on the objects.

For low-power photo-micrographs of white, yellow, pink, or fairly brightly tinted leaves, flowers, or insects, a black background should be used; as it helps greatly to increase the contrasts, and to make the object stand out boldly and

clearly defined in the resulting picture. If a light background is used, careful attention must be paid to the shadows, lest they are too heavy. This trouble, however, is easily overcome with a little manipulation, and the aid of a large sheet of white cardboard to reflect the light back on to the side of the picture where the shadows are deepest. With a little practice, most beautiful effects of light and shade can be produced; and an object with a delicately gradated shadow, makes a far more pleasing and truthful picture than one surrounded by a hard, glaring, white background.

Artificial light will be found to be the best source of illumination for photographing objects by transmitted light. Either oil, some form of gas, or electric light may be used; and very satisfactory results can be obtained with a good round-wicked paraffin oil lamp, or an incandescent gas outfit, such as is sold for use with the optical lantern. Objects to be photographed by transmitted light must, of course, be transparent or semi-transparent,

Lily of the Valley, × 4.

and they may be mounted on glass slips in the same manner as specimens are mounted for microscopic examination.

In taking a low-power photo-micrograph by transmitted light, the object is placed in position on the carrier having a central hole, and this hole must be accurately central with the lens of the camera. The illuminant is placed at the back of the object-holder, and in such a position that the whole field of view is seen to be evenly illuminated on the focussing screen of the camera.

A condenser, if possible, six inches in diameter, should be placed between the source of illumination and the object-holder, so as to concentrate the rays of light upon the specimen. This must also be carefully centred, or the field will be unevenly illuminated. Much time and labour will be saved, by constructing a tin-lined box to carry both condenser and illuminant in their properly centred positions. If it is made to push into grooves on the base board, which will centre it, and is well ventilated, this box will be found a great

help; and if the condenser and light have, in the first place, been properly centred and fixed within it, a permanent saving of time and trouble will have been accomplished.

A sheet of very fine ground-glass may with great advantage be interposed between the back lens of the condenser and the source of illumination, as by this means the light will be more evenly distributed, and much of the dangerous heat cut off from the lens. Great care must be exercised in regulating the light, so that the back lens of the condenser does not become overheated, or it will probably crack.

All plates used for photo-micrography must be backed, to prevent halation, and a plate of moderate speed, and as free from "grain" as possible, should be selected. Some form of isochromatic plate must always be used, as it is of the greatest importance in all branches of photo-micrography that the colour values should be rendered as accurately as possible. I have used for years, with uniform and unvarying success, Messrs. B. J. Edwards' isochromatic medium and instantaneous

plates. They are wonderfully free from "grain," which is of the utmost importance to the photo-micrographer, are easy to work, and may be developed with any of the developers in general use, such as Pyro, Hydrokinone, Metol, Ortol, etc.; moreover, they are sent out ready backed. From long experience, I can recommend them to the novice for all branches of photo-micrography; though, after all, the choice of a photographic plate, like the choice of a cigar, is largely a matter of individual taste; and as there are several brands of isochromatic plates on the market, the tyro might do well to give them all a trial, until he finds the one with which he can obtain the best and most uniform results. By this means, he will be able to select a series of plates, each the most suitable for a particular subject; for no single plate can give the best rendering of *all* the varied subjects which come under the head of photo-micrography.

The length of exposure required to produce a good negative depends entirely

upon the lens, stop, and plate used; on the actinic power of the light employed, and the subject to be photographed. As some sort of guide, we will suppose that we wish to photograph part of a spray of lily of the valley, with a magnification of about four to six diameters, by reflected daylight. With a fairly strong north light, a rectilinear lens working at *f*/16, and an Edward's backed instantaneous isochromatic plate, an exposure of forty to forty-five seconds should be ample. It is better to slightly over than under expose the plate, if not quite certain of the exact amount of exposure required.

Suppose we wish to obtain a photograph, by transmitted light, of a small insect mounted in Canada balsam, and magnified ten or twelve diameters. If the object is fairly transparent, the light (say a good round-wicked paraffin lamp) properly centred, and the plate used an Edwards' instantaneous isochromatic, an exposure of eighteen to twenty or perhaps twenty-five seconds, should produce a satisfactory negative, full of detail and gradation.

CHAPTER II.

MEDIUM AND HIGH-POWER PHOTO-MICROGRAPHY.

A THOROUGH knowledge of medium and high-power photo-micrography is an absolute necessity to the medical student and practitioner of to-day, to enable them to keep an accurate and faithful record of their pathological and bacteriological observations. To the biologist, and indeed to all who are interested in natural science, photo-micrography is practically indispensable; for with its aid, they are now able, in a few moments, to obtain absolutely truthful and perfect representations, of objects which formerly required the most exact and skilled draughtsmanship to delineate.

The magnification in medium-power photo-micrography ranges from about four-

teen or sixteen diameters, to five hundred and fifty; and in high-power work, from six hundred to two thousand or more diameters.

A microscope stand suitable for photo-micrographic work should be as rigid as possible when placed in a horizontal position, should be fitted with a fine-adjustment milled head for focussing with the higher powers, and with rack and pinion coarse-adjustment for focussing with the low-power objectives; it should also have some form of mechanical sub-stage, to carry the sub-stage condenser, etc. The moderate priced microscopes designed for medical and science students, made by Messrs. R. & J. Beck, Ltd., of 68, Cornhill; Messrs. Chas. Baker, of 244, High Holborn; and Messrs. Watson & Son, 313, High Holborn, will be found to meet these requirements; and the pages of the catalogues of these old-established and famous firms will be found to contain descriptions of microscopes at prices to suit all purses.

I would strongly urge upon the novice

who does not already possess a microscope, the wisdom and advisability of devoting the major portion of his initial expenditure to the purchase of a really good and efficient microscope stand; otherwise, he will find it impossible to obtain high-class results in microscopical observation, or in taking photo-micrographs. A good instrument will last a lifetime, and the additional apparatus and lenses can always be purchased as they are required. A very good outfit for the beginner, consisting of microscope, two objectives, eyepiece, and substage condenser, can be purchased for about six or eight pounds from any of the firms mentioned.

For medium and high-power photo-micrography a stand to carry the microscope, camera, and lamp, not unlike that already described for low-power work, will be required, and can be made at home for a few shillings.

As the camera to be used for this branch of photo-micrography must not only have a length of bellows exceeding at least thirty inches, but must also gain its greatest

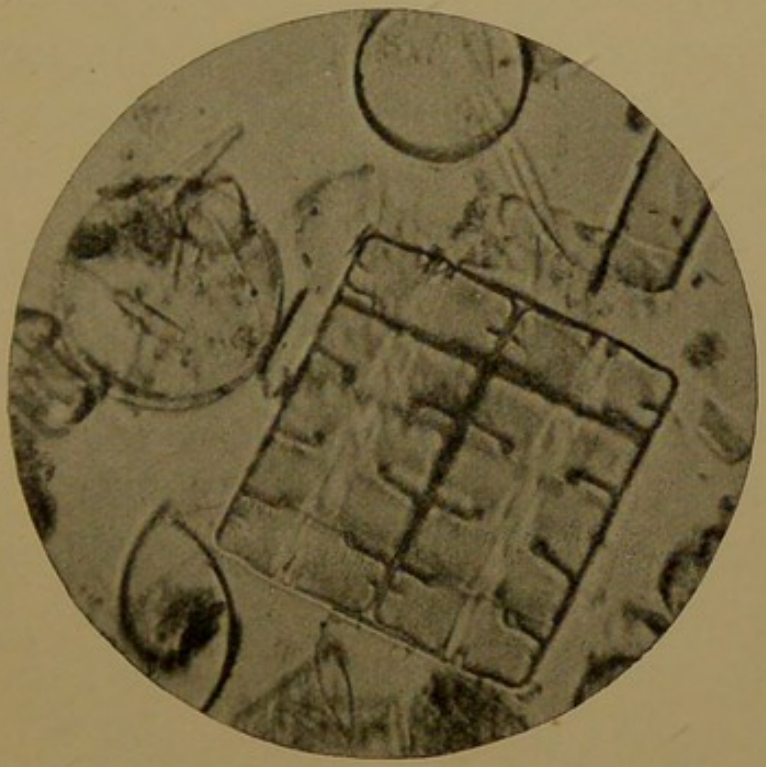
extension by a backward, and not by the usual forward movement, it may with advantage be also made at home; the simplest plan being to procure a second-hand square-bellows quarter-plate camera, to which new bellows of the required length can be easily fitted.

The pine or deal base board should be a foot longer than the one used for low-power work, to allow for the greater length of camera bellows, and quite three-quarters of its length should be devoted to the stage along which the camera must be able to travel. Both the back and front of the camera are attached to the stage by clamps, fitting in a groove which runs down each side of the stage, and enables the camera to be pushed backwards and forwards, and fixed into position.

The microscope, when placed in a horizontal position on the base board, will probably be found to have the body-tube a good deal below the lens-flange of the camera, and therefore a stage will have to be made to raise it into the proper position, with the body-tube exactly in

the centre of the round opening for the lens in the front of the camera. This stage should be attached to the base board by a "screw-down" pin, so that it can be turned round when necessary.

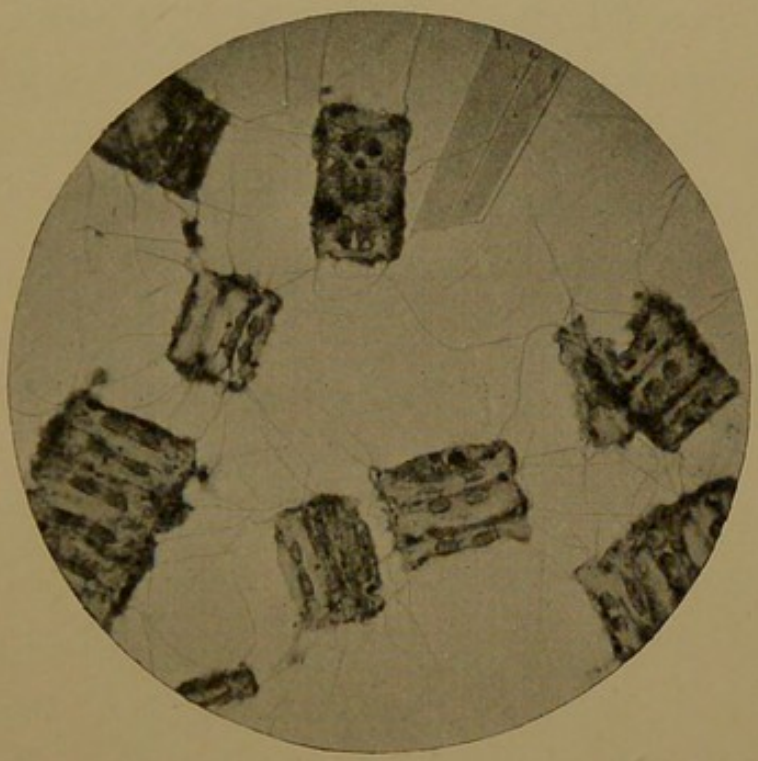
When the stage has been properly adjusted and fastened on to the base board, the microscope with a low-power objective attached (the one-inch will do), and the eyepiece removed, is placed upon it and carefully centred, so that a perfect circle of light is seen upon the middle of the ground-glass focussing screen of the camera. On this being successfully accomplished, two pieces of wood should be screwed on to the stage, one on each side, against the foot of the microscope, so as to make an accurately centred bed, into which the microscope can slide, and thus be always ready in position for work. A third piece of wood should be fastened across the two side pieces to act as a toe-cap, to keep the microscope rigid. Much time and after-trouble will be saved if the lamp is carefully and accurately centred in the same way. If, in the first



A Diatom from the Philippine Islands, × 510.

instance, this centring of lamp and microscope is carefully carried out, there will be no after-trouble or waste of time, as they will always slide into their proper positions at once.

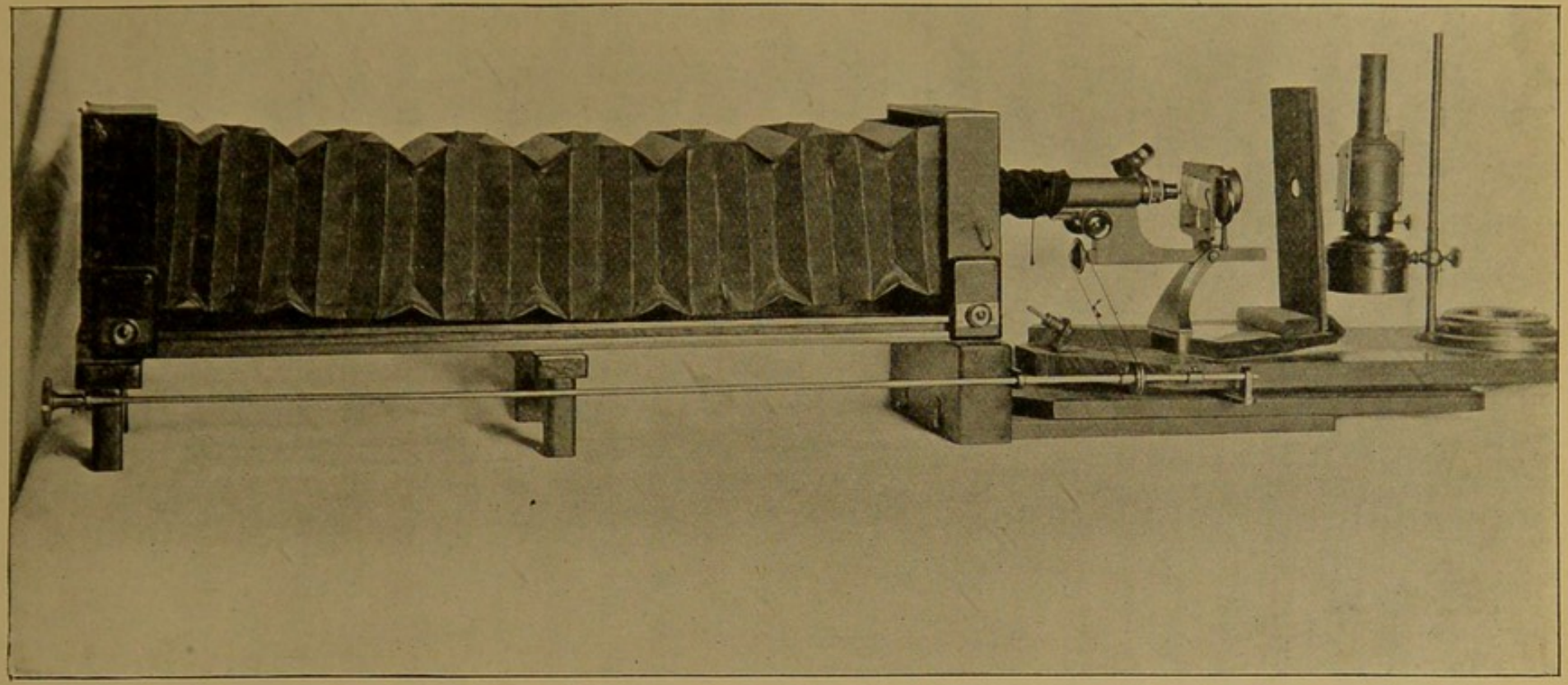
On careful examination, the disc of light on the ground-glass screen of the camera, will be found to have an inner and brighter ring of light, which is due to reflection within the body-tube of the microscope. Though this bright ring of light is not always noticeable upon the focussing screen, it makes itself unpleasantly visible on the negative. This trouble can be easily overcome by making a velvet and cardboard tube to fit inside the body-tube of the microscope ; or such a tube can be purchased from the scientific instrument makers for a small sum. The use of a velvet tube is very much better and safer than coating the interior of the body-tube with some dead black compound; for sooner or later, particles of the paint will dry and crack away from the metal tube, and probably fall upon the back lens of the objective, spoiling



Chactoceros Armatum, × 510.

the definition, and doing no good to the lens.

A focussing rod capable of being connected by means of a silk cord to the fine-adjustment of the microscope, must be mounted on the right-hand side of the base board. The rod itself should be sufficiently long to reach from the camera end of the base board to a little beyond the coarse-adjustment of the microscope. It may be made of brass or iron, and can be mounted on two brackets similar to those used for roller-blinds; one bracket being fastened flush with the camera end of the base board, and the other beyond the coarse-adjustment of the microscope. The rod must be fitted with two milled heads; one to act as a handle, fastened at the camera end, and the other, which must have a groove round it, must be fastened on the rod exactly opposite the fine-adjustment milled head of the microscope. A fine silk cord should be rubbed on a piece of resin, to make it "bite," and then be fastened into a loop to connect the fine-adjustment of the microscope

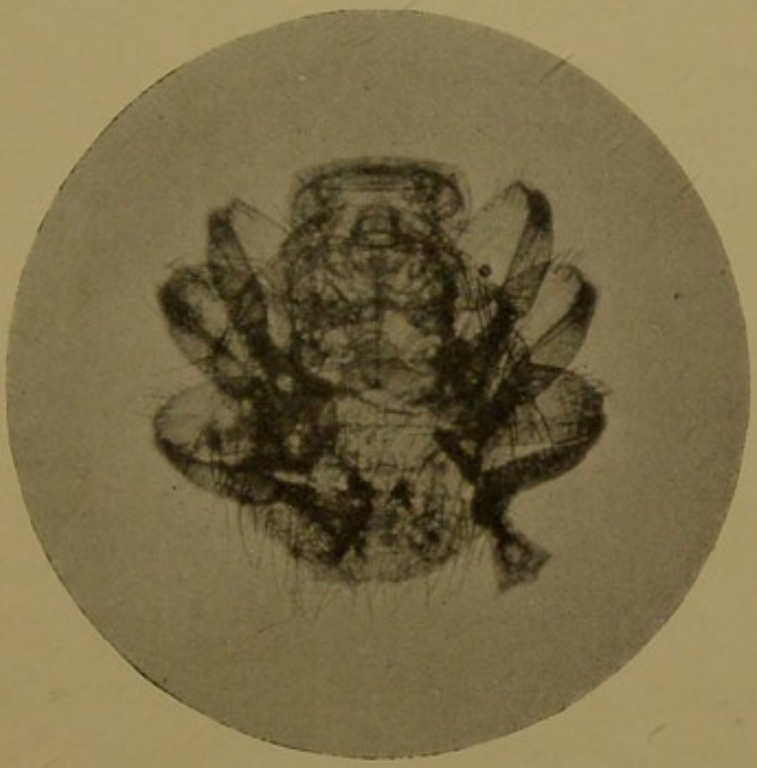


Camera and Microscope in Position for Taking a Medium-Power Photo-Micrograph.

with the milled-head on the focussing rod. On no account must this loop be so tight as to put the least strain upon the delicate fine-adjustment, or it will very quickly be ruined.

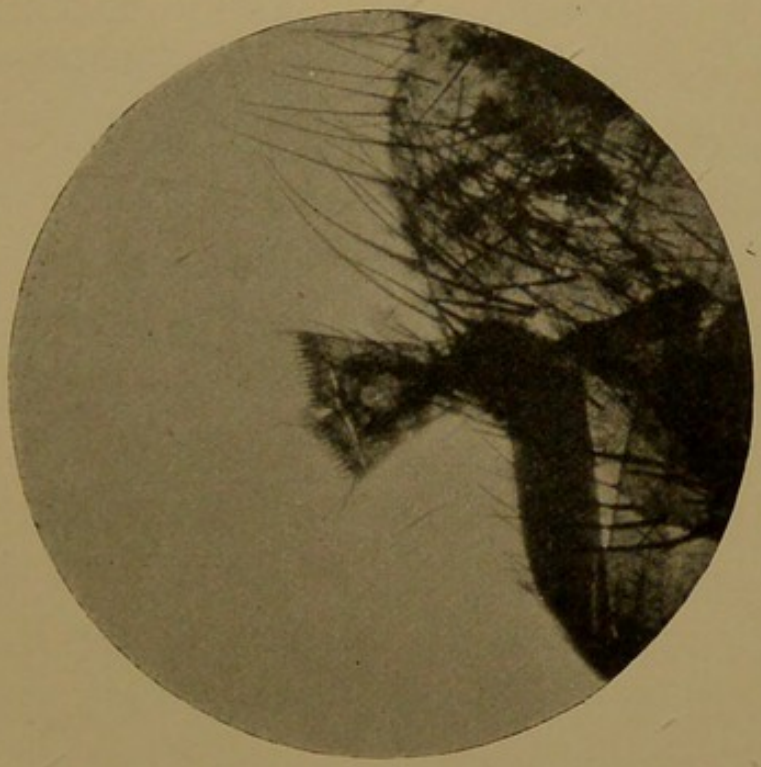
The most handy and convenient form of shutter for cutting off the light while placing the dark slide in the camera, is a piece of stout cardboard mounted on an upright, something like a railway signal. This can be placed between the object-glass and the specimen when working with moderate magnifying powers, and behind the sub-stage condenser, when the higher objectives are being used.

The microscope tube may be conveniently connected with the front of the camera by means of a velvet-covered tube ending in a hood. The tube should be capable of being screwed on to the lens-flange of the camera, and should fit over the body-tube of the microscope; the hood must be fastened with a piece of cord, effectually preventing any extraneous light from entering the camera to fog the plate.



BEE PARASITE (*Braula cœca*), × 25.

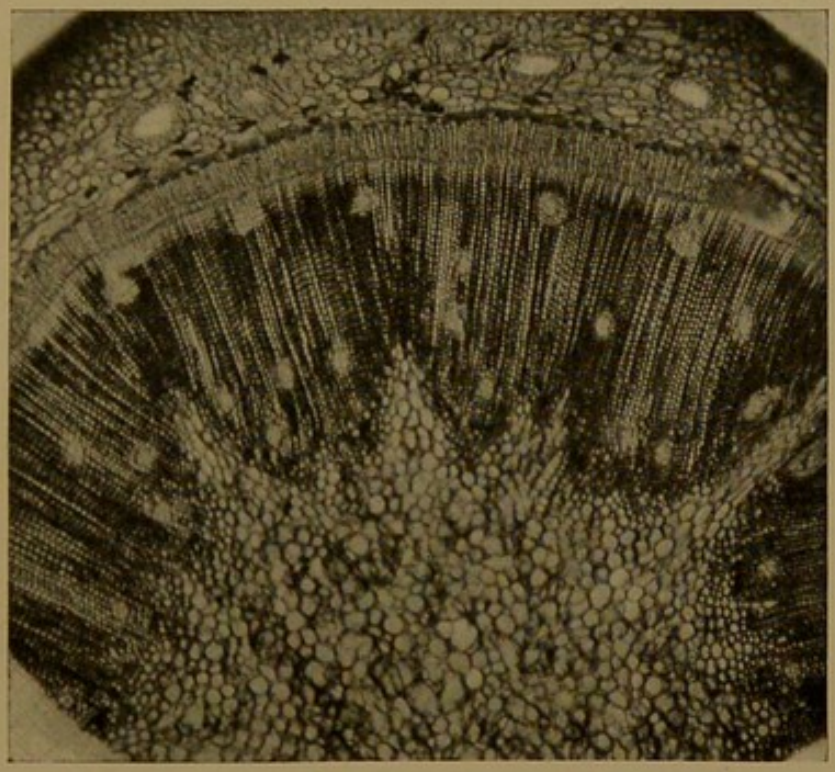
Medium-power photo-micrography should be carefully worked at and thoroughly mastered by the novice ere he attempt to try his "'prentice hand" at the trials and difficulties of high-power work. To essay high-power photo-micrography ere having thoroughly mastered the technic of medium-power work, is to court disaster and the speedy abandonment of a most fascinating branch of photography. There is, indeed, much to be said in praise of medium-power photo-micrography, for its broad range of magnification (fourteen or sixteen diameters to about five hundred), opens up a wide and practically inexhaustible field of work. With its aid, a permanent record of the interesting life histories of all the micro-fungi can be made, to mention one branch of botany alone; while zoology will be found to teem with interesting objects, only requiring a medium magnification to reveal their beauties. Nicely prepared and stained sections of plant ovaries, stems and leaves; parts of insects, such as the eyes, wings, antennæ, feet, stings, etc., and the various



Foot of Bee Parasite (*Braula cæca*).

small and interesting parasitic insects, will be found to yield striking and not too difficult subjects for medium-power photo-micrography.

Suppose the novice wishes to take a photo-micrograph of the interesting dipterous parasite of the bee (*Braula cæca*), showing the entire insect. To do this, the microscope is placed in position on the stand, the one-inch object-glass attached, the eyepiece withdrawn, and the velvet tube inserted in the body-tube of the microscope in its stead. The tubular hood is fastened so as to connect the microscope and camera; and the latter is drawn out to give a bellows extension of, say, fifteen inches, which will, roughly speaking, give a magnification of about twenty-five diameters on the focussing screen. The lamp is lighted and carefully centred, so that the whole field of view on the focussing screen is brightly and evenly illuminated. The specimen is then placed on the microscope-stage, arranged in position, and roughly focussed with the rack-and-pinion coarse-adjustment. Five or ten minutes

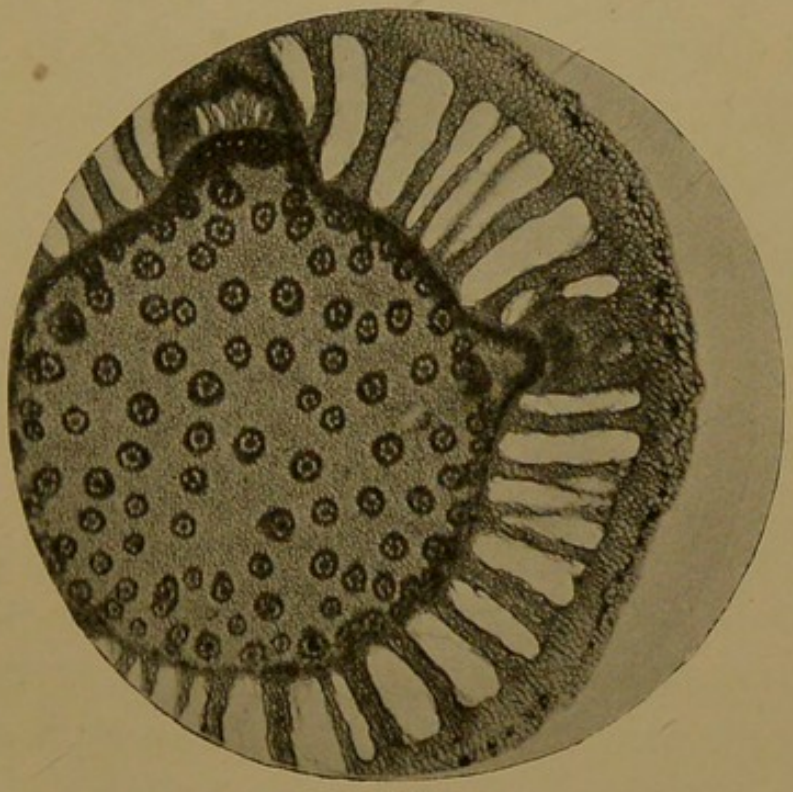


Stem of Larch (Transverse Section), × 59.

must be allowed to elapse before the final focussing of the object, so as to allow for expansion or contraction of the apparatus, due to heat. A sheet of plate glass is now substituted for the ground-glass screen, and the final and critical focus obtained with the aid of the focussing-glass and the focussing-rod connected to the fine-adjustment of the microscope.

Care must be taken that the focussing-glass is accurately in focus on the plate-glass screen—that is to say, it should sharply define the "grain" of a scratch made with a diamond or file, on the surface of the screen. Some little difficulty may at first be experienced in seeing the object with the focussing-glass, which is generally due to the eye being placed too close to the focussing-glass, or to the novice endeavouring to look *through* rather than *on to* the plate-glass screen; but a little practice and manipulation will soon put this trouble right.

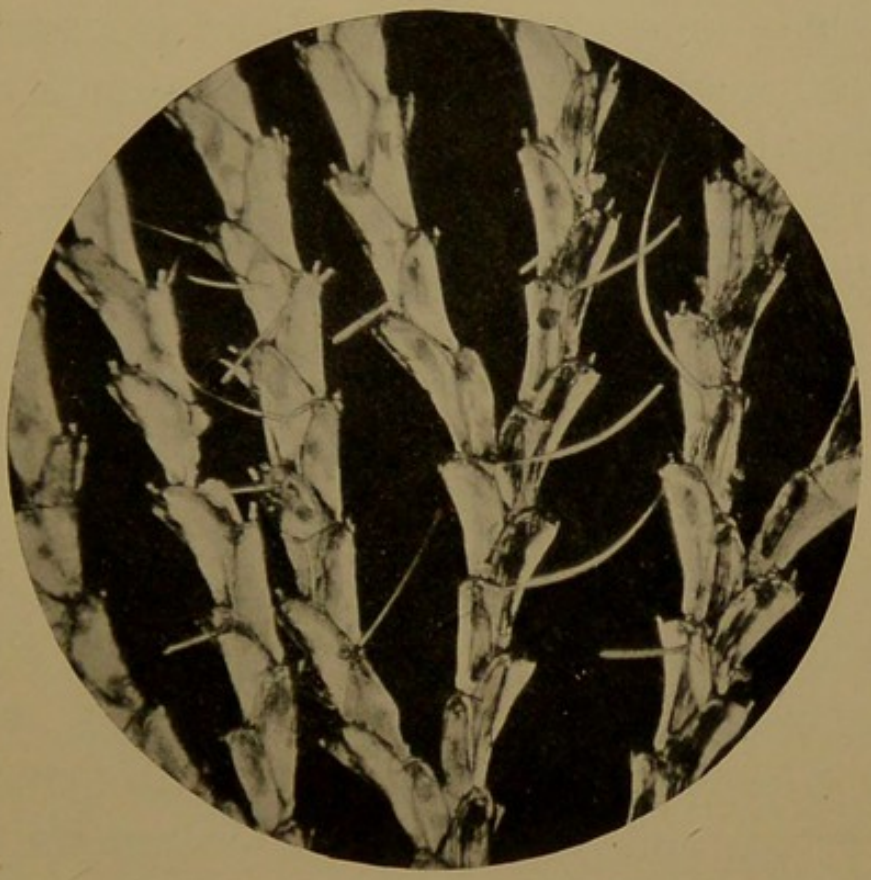
Having obtained a critically sharp image of the bee parasite, the card, which does duty as a shutter, is placed in front of



RHIZOME OF *Juncus lamprocarpus* (TRANSVERSE SECTION), × 16.

the lens to cut off the light, and the plate-carrier inserted. With a good single-wicked microscope-lamp, the edge and not the breadth of the flame being used, twenty-five to thirty seconds exposure with an Edwards' isochromatic instantaneous plate (backed), should be sufficient to yield a brilliant negative, full of detail and gradation.

A satisfactory negative, showing the entire parasite, having been obtained, it will be as well to take another photo-micrograph with a slightly higher magnification, which will show more clearly the characteristic shape of the foot of this curious little insect. With this end in view, the velvet tube is withdrawn, and the No. 1 eyepiece inserted, the camera and microscope again connected, and the camera bellows drawn out to give an extension of twenty inches between the eyepiece of the microscope and the focussing screen. This additional length of bellows in combination with the No. 1 eyepiece, will bring the magnification up to about seventy-six diameters; which will be found sufficient to give a

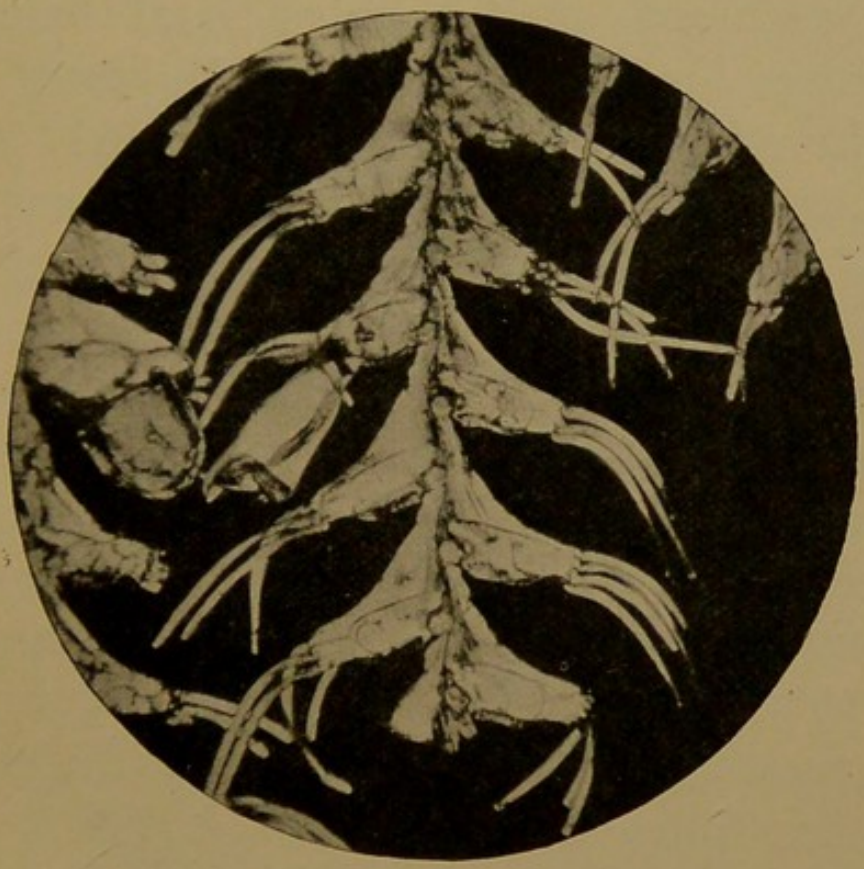


Stirparia glabra (Australian Bryozoon), × 25.

Example of dark ground illumination effect, produced by Spot-lens attached to sub-stage, and Lieberkühn attached to objective.

general view of the characteristic shape and structure of the parasite's foot. The increased magnification will somewhat diminish the amount of light, therefore a slightly longer exposure will be necessary—about forty-five to fifty-five seconds will probably be sufficient.

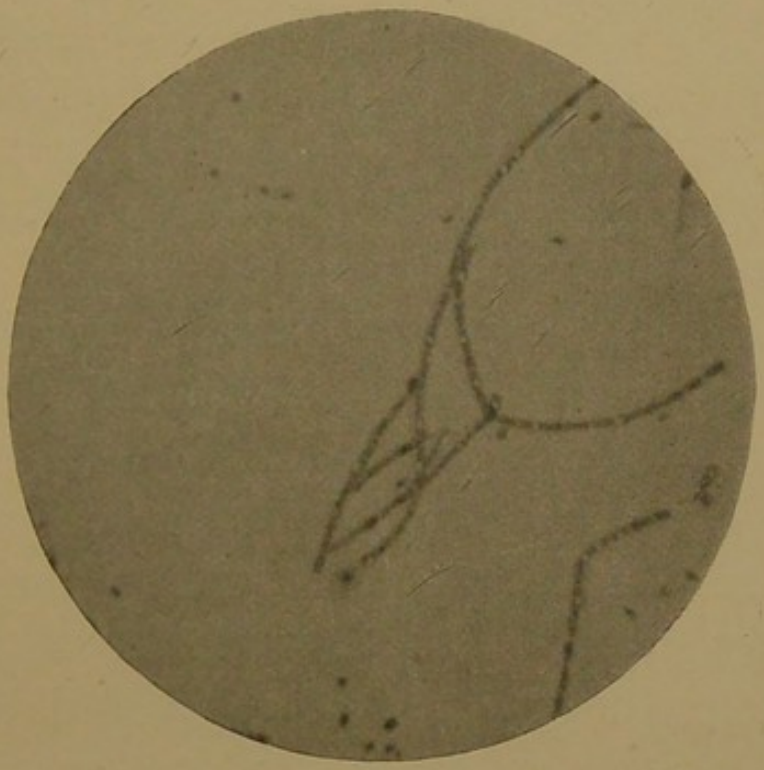
To ascertain how many diameters an object is magnified on the focussing-screen of the camera is not a very difficult matter, though it requires some care. If apochromatic objectives and compensating eyepieces are used, the calculation will be simple, as in the best apochromats each objective is of an accurate focus. Place an object on the microscope stage, screw on a one-inch (24 mm.) apochromat objective, and place a six compensating eyepiece in the microscope tube. Now draw out the bellows of the camera so that the ground-glass screen is 10 inches from the front lens of the eyepiece, which will give a magnification of 63 diameters. Lengthen the bellows to 20 inches; 20 divided by 10 gives a quotient of 2, $10\overline{)20}\ 2$ and the original magnification of 63



BICELLARIA GRANDIS (VICTORIA), × 25.
Example of dark ground illumination.

diameters becomes 126; or, $\frac{63}{126}$, and so on for every extra 10 inches of bellows.

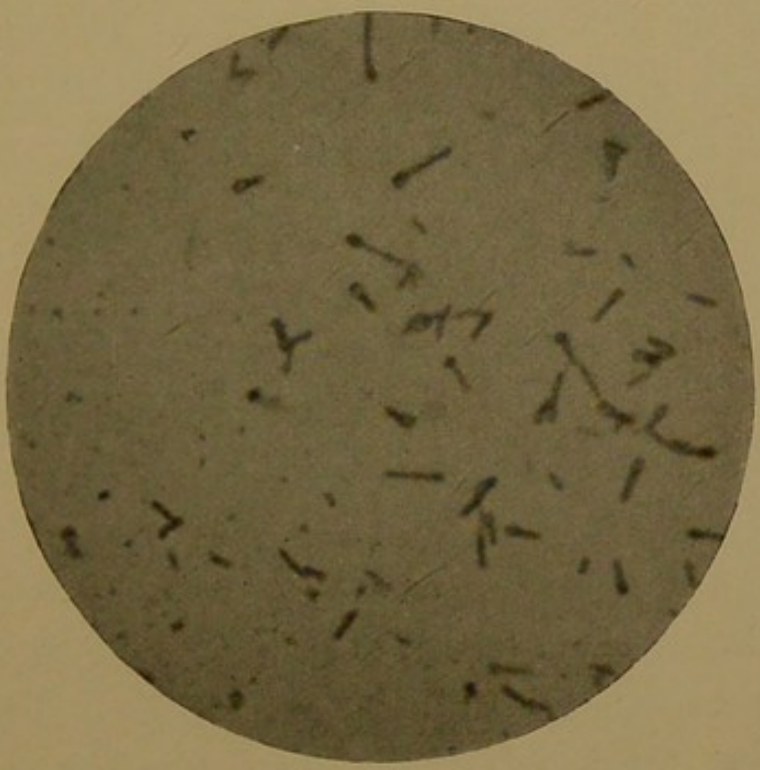
Where achromatic objectives are employed, or there is any uncertainty about the accuracy of the stated focal length, a stage micrometer must be used in conjunction with a rule divided into inches and tenths. A stage micrometer is a piece of glass carefully ruled into hundredths and thousandths of an inch. Place the micrometer on the microscope stage in lieu of the object, and draw out the camera bellows so that the focussing screen is 10 inches from the eyepiece. Focus two of the micrometer lines sharply on the screen, and then with a pair of compasses measure exactly the distance the two lines are apart, and divide it by the distance known to exist between the two lines on the stage micrometer; thus supposing one one-hundredth of an inch when marked on the focussing screen measures one inch and three-tenths, the magnification will be one hundred and thirty diameters. A note should always be made of the magnification used in taking



Bacillus Anthracis (Sporing), × 1200.

each negative, as it greatly enhances the value of the picture, besides being useful for future reference.

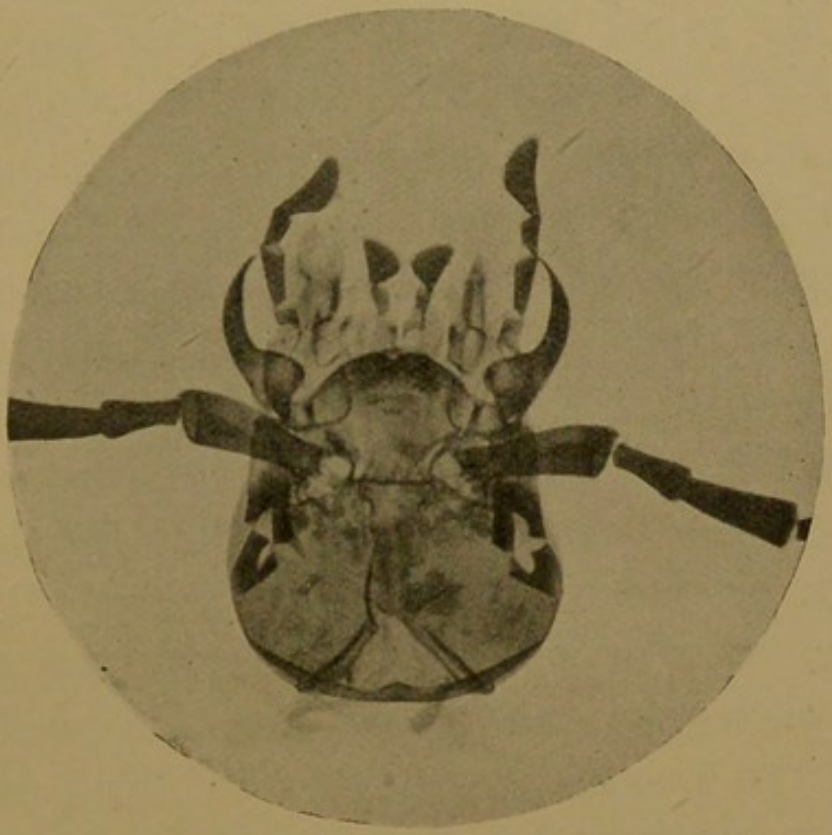
With the increase of magnification, there is necessarily a continual diminution of light and increase of exposure, therefore as the novice passes, step by step, from medium to high-power photo-micrography, he will find that a stronger source of illumination is required than the ordinary microscope lamp, which has hitherto been such a faithful friend. Prolonged exposure must always be avoided to the uttermost, if uniformly good results are to be obtained. Vibration, fuzziness due to the alteration of focus by the expansion or contraction of different parts of the apparatus during exposure, and a general dulling of the image, are three common evils attendant on prolonged exposure, quite sufficient to enforce the absolute necessity for a brilliant source of illumination, sufficiently rich in actinic power to reduce the length of exposure to a minimum. The great objection to the use of incandescent gas is, that when



Bacillus Tetani (Spores), × 1200.

"critical light" has to be used, the uneven appearance of the mantle is of course added to the picture. Therefore, the best form of illumination for high-power work will be found to be either limelight or acetylene gas. Personally I prefer acetylene to the limelight, as it is much cooler, and if properly manipulated, gives an intensely bright white light. For bacteriological and critical work, it is the light *par excellence*. I can strongly recommend Mr. W. Tyler's "Dreadnought" generator for photo-micrographic work; it is absolutely safe, clean, simple in construction, and very portable, and is inexpensive. I am not in any way connected with Mr. Tyler's business, and what I have stated above is simply a statement of my personal experience in using his patent generator, after having tried several others on the market.

The production of successful photographs of bacteria, is the highest attainment in photo-micrography, requiring at once great manipulative skill and patience.



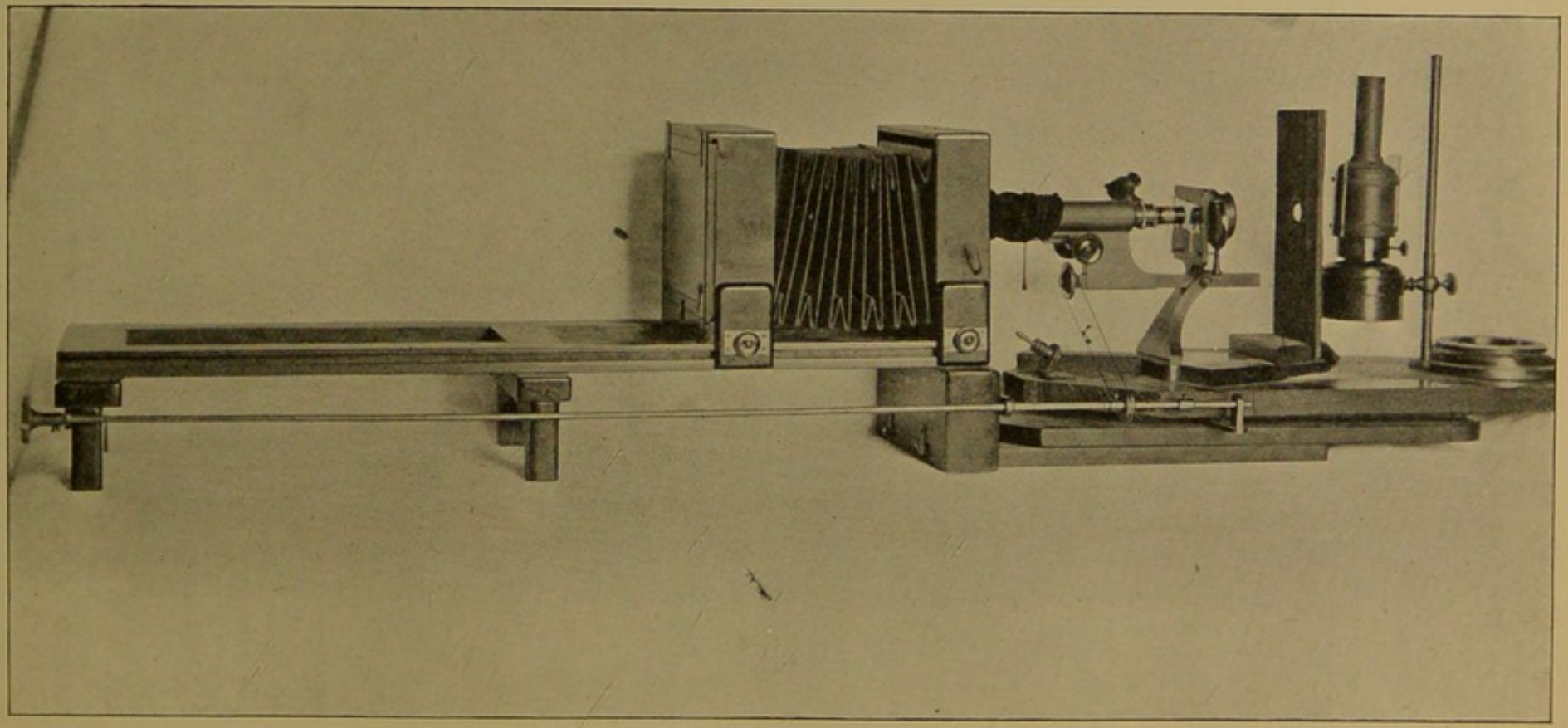
HEAD OF SOLDIER BEETLE, × 20.

So minute are these microscopic organisms that it is only with the very highest magnifying powers they are revealed; therefore the novice must be prepared for many disappointments and must make up his mind that every failure shall be for him a lesson and stepping stone to ultimate success; otherwise, he will soon grow disheartened and abandon the attempt. The greatest care and precision is required in centring the light and sub-stage condenser, and in focussing the bacteria upon the plate-glass screen; and it must always be borne in mind, that the least shock or jar during the exposure of the plate, will utterly spoil the photograph. The plate-carrier must be placed in position with the greatest care, and its sliding panel drawn out smoothly and evenly; otherwise, on development, the image will be found to be out of focus. The best results, in most cases, will be produced by using only an extension of ten inches of camera bellows, in conjunction with a fairly high eyepiece. A crisp, sharp negative obtained in this way will always bear considerable

after-enlargement, so that ultimately a magnification of three thousand or even four thousand diameters, can be satisfactorily obtained. That this re-enlargement of the negative is the method employed by the veteran photo-micrographer, Dr. Van Heurck, is sufficient recommendation for its adoption.

Extremely difficult though this branch of photo-micrography is, it can, with patience and experience, be mastered. Set to work with a fixed determination to conquer all difficulties, and your labours will be crowned with success.

Bacillus Tuberculosis in Sputum, ×1200.



Camera and Microscope in Position for Taking a High-Power Photo-Micrograph.

Part II.

CHAPTER I.

DEVELOPING THE NEGATIVE, PRINTING, ETC.

If you wish to succeed, and to be able with certainty to produce uniformly good results, always use the same brand of plates and the same developer. Experience will teach you that every brand of photographic plate on the market has its own peculiar little idiosyncrasy, which must be mastered ere certainty of perfection can be reached; and this is also the case with most developers. The shortest route to success is to stick to one brand of plate for each particular class of subject, and, say, two or three developers, and to thoroughly master their peculiarities and possibilities.

The plate used for all branches of photo-micrography should be an isochromatic one, and *must be backed*. For developers—

Pyro-soda, or Pyro-ammonia, Hydrokinone and Glycin will be found the most useful.

For producing brilliant negatives, full of delicate gradation and detail, there is nothing to beat Pyro-soda, or, in careful hands, Pyro-ammonia; but the latter is apt to stain both negative and fingers to a very disagreeable degree, if carelessly employed. Beautiful results can be obtained with the following pyro-soda formula, which is also specially recommended by the makers for use with Edwards' isochromatic plates:

SOLUTION A.

Pyrogallic acid . . .	1 oz.
Metabisulphite of soda . .	1 „
Water	80 „

Dissolve the metabisulphite of soda, and then add the pyro.

SOLUTION B.

Carbonate of soda (washing soda)	12 oz.
Sulphite of soda	4 „
Water	80 „

To develop, mix equal parts of solutions

A and B. In warm weather and with a full exposure, add 4 or 5 minims of a 10 per cent. solution of potassium bromide.

For producing very hard negatives with sharp contrasts, some form of hydrokinone developer should be used. The following formula is a general favourite, and will be found a very useful one :

SOLUTION A.

Hydrokinone . . .	160 gr.
Potassium bromide . .	40 „
Citric acid	60 „
Water	20 oz.

SOLUTION B.

Sodium hydroxide . .	160 gr.
Water	20 oz.

To use, take 6 fluid drachms of solutions A and B, and fill up to 16 drachms with water. If the plate is thought to be over-exposed, add from 5 to 10 minims of 10 per cent. solution of potassium bromide. Glycin gives most perfect gradation, and its great advantage over Pyro or Hydrokinone is that the very finest detail does not get blocked out. The following is the

best formula:—Hot water, 10 oz.; Soda Sulphite, 625 gr.; Pot. Carbonate, 1300 gr.; Glycin, 250 gr. Make up in the order given, adding the Glycin in small quantities to avoid too great effervescence. Then put up in 1-oz. bottles filled to the neck and tightly corked. To use, take 1 oz. of this concentrated developer to 3 oz. of water.

The "hypo" bath, for clearing and fixing the negative, should always be fresh for each batch of plates. Hyposulphite of soda is one of the cheapest chemicals the photographer uses, yet, as a fixing bath for negatives, it is used by many people, over and over again, as if it was a most costly compound, not a particle of which must be wasted. The usual formula for this bath is:

Hyposulphite of soda. . .	4 oz.
Water	20 „

When developing isochromatic plates only a very dull red light must be used in the dark room, and the dish should be covered with a piece of board; the plate

only being examined at intervals, and exposed as little as possible during development.

A photo-micrographic negative requires much fuller development than an ordinary landscape or figure study, and unless this is always borne in mind, the novice will probably often spoil what might have been a good negative.

As photo-micrography may be divided into three branches—viz., low, medium, and high-power work—so the negatives for each division will be found to require longer development and greater care.

To develop the low-power photo-micrograph, make up two ounces of the pyro-soda developer, which will be found an ample quantity for a quarter-plate negative. Take the plate out of the dark slide, and carefully dust it with a soft camel-hair brush kept only for that purpose. The backing may with advantage be next removed with the aid of a sponge, the greatest care being taken that damp or wet fingers, or drops of water, do not come in contact with the gelatine side

of the plate, or blemishes will appear on development. This operation is a difficult one, and, in the case of the ready-backed plates of Messrs. Edwards and several other firms, by no means absolutely essential, as the composition of the backing does not stain the film or interfere with the action of the developer; therefore, I should advise the novice, after dusting the plate, to place it in the developing dish *without* removing the backing. The plate (film side up) is flooded with developer, and the dish covered at once with the piece of board, and rocked steadily to and fro, so that the developer may be kept in constant movement over the negative. After the developer has been allowed to act for one or two minutes, uncover the dish, and examine the negative to see if the high-lights have begun to appear. When the image appears to be fairly visible, take the plate out of the dish, and with a sponge remove the backing. Continue development until the object can be made out on the back of the plate; the negative may then be rinsed

in water, and transferred to the "hypo" bath, where it must remain for at least five minutes after the white deposit has disappeared.

The plate should then be washed in running water for a couple of hours, at the end of which time it may be drained, the superfluous water gently removed with a tuft of cotton wool, and then placed in a dish, and flooded with good clean methylated spirits of wine for five or six minutes, after which it is taken out, drained, and put aside in a warm dust-free place to dry.

The medium-power negative is treated in the same way, save that development must be carried somewhat further, until the image has darkened very deeply on the front of the negative, and is fairly visible (in general outline) on the back of the plate.

Good negatives of bacteria are most difficult to produce, requiring the greatest care and skill throughout the entire process. Owing to their small size, even when magnified up to twelve hundred

diameters, it is practically impossible by the aid of the dim red light of the dark room lamp, to see the image of these minute organisms appearing on the face or back of the plate; and therefore experience alone can enable one to realise when the required amount of density has been gained. Not until the face of the negative has become uniformly black, and the back a deep even tone, should development be arrested. With negatives of bacteria, it is better rather to over than under develop, as, if too dense, they can, after fixing, be easily reduced, and the contrasts considerably increased thereby.

The following bath will be found most useful if used after the negative has been washed a short time, as it helps to get rid of the remaining "hypo," hardens the film, removes developer stains to a very great degree, and often considerably brightens up the negative:

Alum	2 oz.
Citric acid . . .	1 „
Water	20 „

Positives from photo-micrographic nega-

tives should in most cases be made on a paper prepared with a glossy surface, as the more delicate details do not show up so clearly on a matt surface. For daylight printing, P.O.P., or albumenised paper may be used; the latter will generally be found to give the best and most permanent prints, but requires more careful handling and experience. Prints made on either of these papers should be printed much darker than is required for the finished picture, as a good deal of reduction takes place during the toning and fixing.

To make a positive with either of these papers, put the negative, film side up, in a printing frame, and place a piece of the paper face downwards, upon it. If the negative is a good plucky one, print in a fairly strong light (if very dense in direct sunlight), until the print is somewhat over printed. Then, in a subdued light, remove the print and immerse it in a basin of cold water. In a few moments, little silvery-white spirals will begin to ascend from the surface of the print, imparting a milky hue to the water, which must be changed

several times until it remains quite clear.

The print is now ready for the toning bath, which may be made as follows:

Borax	90 gr.
Trichloride of gold . . .	1½ „
Water	10 oz.

Into this bath, which should be kept at a temperature of 60° to 65° Fahr., place the print, face downwards at first, and when a couple of minutes have elapsed, turn it face upwards. Toning will be completed when the print is seen to be the same colour when looked *through* as when looked *at*. It is washed for ten minutes in running, or several changes of fresh, water, and transferred to a fixing bath consisting of:

Hyposulphite of soda . .	4 oz.
Water	20 „

In this bath it should remain for ten minutes, or a little longer, if the paper is very thick; after which a thorough washing, for at least an hour and a half,

must be given, and the print then hung up to dry.

A paper which yields most beautifully uniform results, and which, as it is worked by artificial light, can be employed all the year round, is Eastman's "Nikko" paper, a glossy bromide paper. This paper must be handled in the dark room, a fairly strong orange or canary light being used. A piece of the paper is placed face downwards on the negative, in the printing frame, and then exposed, at a distance of one foot, to the light of an ordinary round-wicked paraffin lamp, or bat's-wing gas burner. From twenty to forty seconds will be found sufficient exposure for an average negative, the frame being kept in motion the whole time, so that the light may pass evenly all over the surface of the negative.

The printing frame is taken back to the dark room, and the piece of "Nikko" paper taken out and placed in a dish of water. When the paper has uncurled and become limp, the water is drained off, and the following developer poured

quickly in an even flood, over the surface :

Amidol	25 gr.
Bromide of potassium . .	5 „
Sulphite of sodium . .	325 „
Water	10 oz.

Rock the dish rapidly and steadily, so that the developer flows evenly over the surface of the paper. In about fifteen seconds, the image will begin to appear, and if the right exposure has been given. development will be completed in about forty-five seconds. When the image appears to be sufficiently developed, pour off the developer, and quickly wash the print for a minute in clean water, before transferring it to the "hypo" bath, which should be a little weaker than that used for fixing plates, and which must always be fresh and clean, or bad stains will result.

Amidol developer does not keep, and must therefore be made up on the day it is going to be used. The best plan is to make up a stock solution of the

sodium sulphite and potassium bromide, and half an hour before using weigh out and add the required amount of amidol. Ten ounces of developer will be found an ample quantity for two packets (twenty-four sheets) of quarter-plate size "Nikko" paper.

After remaining in the "hypo" bath for ten minutes to thoroughly fix, the prints must be taken out and washed in running water for two hours, to eliminate every trace of "hypo."

When the prints have become quite dry, any black marks and lines on the surface can be easily removed by gently rubbing with a tuft of cotton wool saturated in methylated spirit. After the prints have been cleaned, they should be thrown into a basin of clean cold water, and as they uncurl, taken out and placed face downwards on a sheet of plate glass, or a ferrotype plate. A piece of thick, clean, white blotting paper is then placed on top, and a roller "squeegee" passed over the prints to bring them into contact with the surface of the glass or ferrotype

plate. The prints must then be set aside to dry, when they will readily peel off the plate, and be found to have a highly glossy surface, which wonderfully sharpens and brightens up the image.

There are so many excellent brands of lantern plates on the market now, that the novice should have no difficulty in finding one to suit his taste. I have for some time past used nothing but Cadett blue-label lantern plates, as I have always found them carefully prepared with a fine emulsion, very free from grain, and therefore admirably suited for photo-micrographic work; moreover by varying the length of exposure and the developer, a considerable range of tones can be obtained.

In the dark room, the negative, film upwards, is placed in a printing frame constructed for making lantern slides, which can be purchased for a shilling or eighteenpence. A lantern plate is then taken out of the box, carefully dusted, and placed with its film next the negative, and the exposure made to either artificial

or day light; the length of exposure depending upon the source of illumination, the density of the negative, and the speed of the lantern plate. For making lantern slides by contact printing, artificial light is undoubtedly the best, but when a reducing camera has to be employed, as in making a lantern slide from a half-plate negative, daylight may be used with considerable advantage. However, the majority of photo-micrographic negatives are quarter-plate size, enabling the photographer to make his slides by contact.

To obtain perfectly uniform results, the same source and amount of illumination should always be used, and either oil, gas, or magnesium ribbon can be employed; personally I prefer and always use magnesium ribbon.

Where a number of lantern slides have to be made, an exposure board will be found very useful. It simply consists of a deal or pine board, four to six feet long, with lines drawn across it in ink or black paint, at intervals of six inches apart, down its entire length. A groove or slot should

be made at one end, to carry the printing frame.

Supposing a lantern slide is to be made of the bee parasite, and the lantern plate used is a Cadett blue-label. The printing frame containing the negative and lantern plate is placed in position on the board, with a velvet-covered sheet of card placed in front to cut off the light from the spirit lamp; the spirit lamp is placed on the line, which is two feet six inches away from the face of the printing frame, and a piece of magnesium ribbon measuring about one inch in length, which should be held with a pair of pincers or scissors, inserted in the flame of the lamp. The ribbon should be held in the left hand, and as it is placed in the flame the card-board shield in front of the printing frame can be removed with the right hand. The instant the magnesium ribbon ignites, wave it slowly up and down, carefully keeping it the prescribed distance, so that the whole of the plate receives the same amount of illumination. According to the density of the negative, so the inch of

magnesium ribbon must be burnt nearer or farther away. In the case of very weak and thin negatives, a screen of tissue paper should be placed between the source of illumination and the front of the negative, so as to diffuse the light.

Either Pyro, Hydrokinone, or Amidol may be used for developing the lantern plate, the formulæ already given for negatives working well. The hardest contrasts will be gained with correct exposure and the Hydrokinone developer; but care must be taken that the right exposure is given, and that not too much potassium bromide is used, or an unpleasant, greenish-brown colour will be obtained. In some hands Amidol proves somewhat difficult, the image rushing up at first, but ultimately lacking in density; this is no doubt due to improper exposure, as, with ordinary care, most beautiful results can be obtained. Pyro, however, will be found the most useful all-round developer.

Development should be continued until the image begins to appear on the back

of the lantern plate, when the plate must be taken out of the developer, carefully washed, and placed in the "hypo" bath (same strength as for negatives), in which it should remain for at least five minutes. It should then be washed in running water for an hour, or longer if there is no sign of frilling. At the end of the washing, the slide should be held in the left hand under the tap, and the film side very carefully and gently brushed over with a tuft of cotton wool, to cleanse the film of any sediment that may have formed on its surface during washing. This operation requires great care, as the film of a lantern plate is extremely delicate. The plate is then immersed in a bath of clean methylated spirit for five or ten minutes, taken out, its glass back carefully wiped dry, and placed on one side to allow the film to dry. The methylated spirit is used to hasten this operation, as it is most necessary for the slide to be dried as quickly as possible; otherwise, unless enclosed in a dust-proof drying box, particles of dust floating in the air will settle upon

the film while it is wet, stick to it, and spoil the slide.

When it is thoroughly dry, the slide is mounted by binding it, with the aid of specially prepared "binding strips," to another piece of glass, which must be the same size as the lantern slide, and absolutely free from scratches or air bells; a mask of black paper of suitable shape being interposed, so as to prevent the film touching the surface of the cover-glass.

Should a negative or lantern slide, on being taken out of the "hypo" bath, be too dense from over-development, it may be reduced with either a potassium ferricyanide or ammonium persulphate bath; the former greatly increasing the contrasts, while the latter only attacks the denser deposits, and therefore does not destroy the finer details. The ferricyanide bath is made as follows:

SOLUTION A.

Potassium ferricyanide . .	1 oz.
Water	20 „

SOLUTION B.

Sodium hyposulphite . .	1 oz.
Water	20 „

To use, pour a couple of ounces of solution B in a dish and add about a drachm of solution A. If the plate has just been taken from the "hypo" bath, give it a good rinse under the tap; if it has been allowed to dry, it must be thoroughly wetted by soaking the film in water for a quarter of an hour. It is then placed in the ferricyanide bath and gently rocked for a minute or two, then taken out, rinsed under the tap, and examined. If not sufficiently reduced, the process is repeated, care always being taken to wash the plate before examining it so as to stop reduction. When the reduction has been carried far enough, the negative must be well washed in running water for an hour.

The ammonium persulphate bath consists of:

Ammonium persulphate .	16 gr.
Water	2 oz.

The plate must be thoroughly well washed before placing in this bath, as any

trace of "hypo" will tend to destroy its reducing power. The manipulation is the same as with the ferricyanide bath.

When a negative is very thin and lacks sufficient contrast, it may often be very greatly improved by intensification. In ten ounces of hot water dissolve half an ounce of sodium chloride, and add a quarter of an ounce of powdered mercury bichloride, corrosive sublimate—a most deadly poison. Shake the bottle well, and if there is any cloudiness, add a few drops of hydrochloric acid. The bottle should be shaken at intervals several times, and then set aside to allow any sediment to fall to the bottom, when the clear fluid must be carefully poured off into a bottle covered with brown paper to keep out the light. Soak the negative in water for ten minutes, and then place it in a dish, cover with the above solution, and gently rock. In a few moments the black film turns grey, and then pale yellow or white. Wash the negative in running water for twenty minutes, and rub its surface with a tuft of wool. While the negative is washing,

after it has been in the mercury bleaching bath, make up the following solution:

Liquid Ammonia 880. .	20 min.
Water	1 oz.

Place the bleached and washed negative in a clean dish, and pour over it the ammonia solution, and gently rock the dish until the film is thoroughly darkened, and no trace of the bleaching remains visible on either side of the plate. Then wash thoroughly for ten to fifteen minutes and set aside to dry. This process may be repeated, if it is found that the intensification has not been sufficient; though re-intensification is rarely necessary, and not very satisfactory.

CHAPTER II.

PREPARATION OF OBJECTS FOR PHOTO-MICROGRAPHY.

EVERY photo-micrographer should learn how to prepare and mount microscopic objects, as he will find that interesting specimens, which he would like to photograph, are constantly turning up; and with some familiarity of the methods employed in the preparation of microscopical material to aid him, the novice can form for himself an unique and valuable collection of microscopical objects, at a very small outlay. The apparatus required for mounting and preserving need be neither elaborate nor expensive, and can be purchased for a few shillings from any scientific instrument maker. The following is a list of the principal articles required:

1 gross 3x1 glass slips,
½ oz. thin cover-glasses,

1 razor (perferably hollow-ground on one side only),
3 dissecting knives,
2 pairs of micro forceps (one pair straight and one curved),
1 pair of fine scissors,
3 or 4 needles mounted in wooden handles,
1 bundle of pith,
½ lb. paraffin wax,
6 large, deep watch-glasses.

In the way of chemicals and mounting media, the following will be needful:

1 pint methylated spirit (the best),
2 oz. rectified spirits of wine,
2 „ absolute alcohol,
1 „ oil of cloves,
2 „ pure glycerine,
2 „ best turpentine,
1 „ chloroform,
½ „ carbolic acid,
1 „ hydrochloric acid,
1 bottle of Canada balsam (dissolved in chloroform or xylol),
1 bottle of Deane's medium.
1 oz. best gold-size.

For staining, one or two ounce bottles of eosin, hæmatoxylin, acid aniline green, and borax carmine, should be obtained

from the optician. Specimens stained with blue stains should always be avoided, as they never yield satisfactory negatives.

The simplest method of mounting a portion of a leaf, flower, or seaweed, is to thoroughly wash it for a few minutes in a bath made of :

Rectified spirits of wine .	1½ oz.
Distilled water . . .	1½ ,,
Pure glycerine . . .	5 fluid dr.

Transfer the object from the bath to a clean glass slip, and drain off the superfluous fluid. The bottle of Deane's medium having been previously placed in a cup of hot water to melt its contents, a drop or two of the medium is taken out with the aid of a glass rod, and placed on the object. A clean cover-glass is gently warmed in the flame of a spirit lamp, and lowered from one side on to the object in such a way as to drive any superfluous medium in a wave in one direction. The medium is then allowed to set, when that which was expelled from under the cover-glass can be cleaned off with a soft wet rag, the slide carefully dried, and a ring

of gold-size run round the edge of the cover-glass, to prevent any of the medium escaping.

Portions of leaves, and sections of the roots, stems, and seed-receptacles (ovaries) of plants, make very beautiful and interesting objects for photo-micrography, when nicely stained and mounted in Canada balsam. They should be collected in as perfect condition as possible, and while fresh, cut into small pieces about an inch in length, and placed in a jar containing a liberal supply of methylated spirit, which must be changed every day, until it ceases to become discoloured. Sections of the material can then be made.

To cut the sections, the material must be embedded either in pith, or paraffin wax. Cut a piece of pith slightly longer than the stem from which sections are to be made, and split it in half lengthwise. Place the plant-stem between the two pieces of pith, and tie them securely together at one end. Hold the embedded stem firmly in the left hand, using the thumb and middle finger. Take up the

razor with the right hand, and make a drawing cut, using the whole length of the blade, from heel to tip. The razor must be kept well moistened with dilute methylated spirit, and should also be carefully stropped, from time to time, during the section cutting operations. With a little practice, very satisfactory thin sections can be cut from specimens embedded in pith.

For general purposes, paraffin wax will be found very useful as an embedding mass. Alone, it is too hard and brittle, and the high temperature required to melt it would in most cases quite spoil the specimen; therefore, it has to be mixed either with vaseline or lard. A useful embedding mass can be made by taking 8 oz. of solid paraffin wax and 2 oz. of lard, and melting them together slowly, with the aid of gentle heat.

To embed, take the specimen out of the spirit, and gently dry its surface with a piece of blotting-paper or cloth. Place it in a little paper mould or pill-box, in the desired position for cutting the sections,

and then pour into the mould or box a sufficient quantity of melted mixed paraffin to fill it. When the wax has thoroughly cooled and solidified, the box or mould can be broken away, and sections cut with the razor, from the embedded mass. The sections as they are cut, may be placed in a jar of methylated spirit, in which they can remain until it is convenient to mount them.

After the sections have been cut, they must be placed in turpentine to remove the paraffin, and then in absolute alcohol to get rid of the turpentine, when they will be ready for staining.

With some botanical specimens a certain amount of colour remains and refuses to be dislodged, even by a prolonged soaking in methylated spirit; and these, after they have been embedded and cut into sections, must be placed in the following bleaching bath, ere they can be properly stained:

Chloride of lime . . .	2 oz.
Washing soda . . .	4 „
Distilled water . . .	2 pints.

The lime must be dissolved in one pint of

the water, and the soda in the other; and when the two solutions are thoroughly dissolved, they must be mixed together, and set aside for twenty-four hours. The clear fluid is then filtered off into a clean, stoppered bottle, which must be kept in a dark place, or be covered with two thicknesses of brown paper, to keep the fluid away from the light. The sections are taken out of the spirit, thoroughly washed in several changes of distilled water, and then allowed to soak in a quantity of the bleaching fluid for one or several hours. When the bleaching is completed every trace of the bleaching fluid must be washed away, after which the sections may be bottled up in spirit for future use, or be at once stained and mounted.

Sections of stems, roots, and ovaries, when double-stained, make beautiful subjects for photo-micrography. Filter thirty or forty minims of hæmatoxylin, and dilute with one ounce of distilled water. In this solution immerse the sections for ten, fifteen, or thirty minutes. Take the sections out of the hæmatoxylin, and place

them in a very dilute solution of acetic acid in distilled water. Wash away all traces of the acid with distilled water, and then soak the sections in ordinary tap-water for a quarter of an hour. The sections are then placed in the eosin stain for five or six minutes, and thoroughly washed in methylated spirit. They are then placed in absolute alcohol for a few minutes to rid them of all watery moisture (dehydrated), placed on the surface of a watch-glass full of clove oil, in which they remain until they sink and become clear, when they are rinsed in turpentine, and mounted in Canada balsam.

All objects to be mounted in Canada balsam must first be thoroughly freed of every particle of watery moisture, otherwise, in a few days or weeks, the object will become cloudy, and the mount quite useless as a microscopic object. To get rid of the moisture, the specimens are first placed in methylated spirit, which must be changed two or three times at least, and finally into absolute alcohol. They are then placed in oil of cloves to clear them and

free them from spirit, and finally washed in turpentine to expel the clove oil. A glass slip is then cleaned, warmed over the flame of the spirit lamp, and a little convex pool of Canada balsam formed in the middle. The specimen is placed in the balsam, a carefully cleaned and warmed cover-glass is taken up with the forceps, brought in contact with one of the convex sides of the pool of balsam, and carefully pressed down from one side, in such a manner as to expel the superfluous balsam in an even wave.

The slide is then set aside in a warm place, free from dust, to dry, when the excess of balsam can be removed with the aid of a soft rag dipped in chloroform.

Both the hæmatoxylin and eosin are very useful as single stains; the latter staining the sieve-tubes, to be seen in transverse and longitudinal sections of the stem of the vegetable marrow, beautifully. The section is placed in eosin for eight or ten minutes, washed in methylated spirit, dehydrated, cleared in clove oil, and mounted in balsam. In using

the hæmatoxylin as a single stain, no dilute acid bath is used, but the section is transferred from the stain to distilled water, in which it is thoroughly washed; it is then washed in several changes of tap-water to fix and deepen the colour, dehydrated for at least ten minutes in several changes of spirit, cleared in clove oil, and mounted in balsam.

To use borax carmine, place the section in a watch-glass full of the stain for four or five minutes. Wash thoroughly in methylated spirit, and place in an acidulated spirit bath composed of one part hydrochloric acid to five parts methylated spirit, where the section must remain until the colour becomes a bright scarlet. The section is then washed in several changes of methylated spirit, dehydrated, and mounted in balsam.

Many very small insects, such as baby caterpillars, ants, fleas, and red spiders, may be mounted for photographing with the one-inch object-glass. They should be killed with chloroform, and placed in methylated spirit, which must be changed

several times. They can then be mounted in balsam, or stored in spirit, or turpentine, which slightly increases their transparency. Some insects will be found to require treating with a 10 per cent. solution of caustic potash in distilled water, to soften them and expel the contents of the abdomen.

Portions of sea-weeds bearing the reproductive organs, are beautiful objects for the photo-micrographer. They should on no account be allowed to dry, or they will shrivel and give a false idea of their real appearance. While quite freshly gathered, and in good condition, the fronds of the sea-weeds must be examined with a fairly strong hand-magnifier. The portions selected for mounting are carefully washed in clean water, placed on a glass slip, and mounted in Deane's medium.

Nearly all animal tissues can be hardened and preserved in methylated spirit or absolute alcohol. A large quantity of spirit should be used in comparison to the bulk of tissue placed in the jar or bottle, and must be changed once in every twenty-

four hours, during the first three or four days. Eosin, hæmatoxylin, and picro-carmine, will be found the most useful stains; and Canada balsam and Farrant's medium the best mounting media.

Of such great importance in modern science is the study of bacteriology, and so intimately has it become associated with the practice of high-power photo-micrography, that a knowledge of some of the methods employed in the preparation and staining of these minute micro-organisms is absolutely essential to the earnest student of this most fascinating branch of photography. Only the barest outlines can be given here, for anything approaching a detailed description of the organisms, their life-histories, cultivation, and preparation for microscopical examination, would more than fill a volume six or eight times the size of this text-book. The student who wishes to gain some insight into the mysteries of the cultivation of bacteria cannot do better than purchase a copy of Dr. W. Migula's "Introduction to Practical Bacteriology."

The stains used in bacteriology differ in many respects from those employed for ordinary microscopical purposes, in most cases being much more powerful in their action; and most of these belong to the so-called basic aniline colours. The principal stains employed are methyl-violet, gentian-violet, methylene-blue (not much good for photo-micrographic work), fuchsin, Bismark-brown, and Vesuvian; they are used in either saturated watery solutions, or in dilute alcoholic solutions. Carbol-fuchsin will be found a most useful stain, in preparing bacteria for photo-micrography.

All specimens treated with these basic aniline dyes, must be mounted in Canada balsam dissolved in xylol, as chloroform has a deleterious action upon the stain, extracting it from the bacteria; Deane's medium may frequently be used with great advantage for mounting stained bacilli. Whether balsam or Dean's medium are used in mounting, a ring of gold-size must be run round the edge of the cover-glass, to seal it, and to prevent the cedar oil

used with the immersion lens getting underneath and spoiling the mount.

As an introduction to the microscopic appearance of these organisms, and for practice in staining and photographing them, the student cannot do better than draw upon a natural and prolific source which is always at his disposal—to wit, the white "fur" which is always present on the teeth; and the various forms of bacteria to be found in an infusion of hay.

Place a single drop of water on a thin cover-glass, and diffuse therein a very small quantity of the "fur" freshly removed from the teeth, so that the thinnest possible film of the material is formed on the surface of the glass. Set the cover-glass aside to dry, in a situation absolutely free from dust. When perfectly air-dried, pass the cover-glass, *bacteria-covered side upwards*, at a moderate speed, three times through the flame of a spirit lamp, or a bunsen burner, which will effectually fix the organisms, and permit of their further manipulation.

Spread upon the bacteria-covered side of the cover-glass a few drops of carbol-fuchsin, and allow it to act for a few minutes, after which, the cover-glass must be well rinsed in distilled water, to free it from superfluous stain, its clean side dried, and the preparation examined in water. If the bacteria are not deeply coloured, repeat the process, and slightly heat the stain. Should the preparation be over-stained, part of the colour can be got rid of, by washing in absolute alcohol, or oil of cloves. The preparation, after being stained and dried, may be cleared with a drop of turpentine, or oil of bergamot, and mounted in xylol-balsam. To distinguish readily the *bacillus buccalis* in the "fur" of the teeth, proceed as above, substituting iodine for the carbol-fuchsin.

Steep some dry hay in a small quantity of water, for four or five hours, when the infusion may be poured off and diluted. Plug the flask or test-tube containing the fluid with a big tuft of cotton wool, and allow the contents to gently boil for half to three-quarters of an hour. Let the

flask remain undisturbed for twenty-four to forty-eight hours, at the end of which time a delicate scum will be noticed on the surface of the fluid. Remove the cotton wool plug, transfer a small quantity of the scum on to a cover-glass, and dry, stain, and mount in the manner described above. The long cylindrical rodlets which will then be seen, are the *bacillus subtilis*. The *bacillus subtilis* may also be stained with chlorzinc-iodine, which stains the bacteria a brownish-yellow colour.

To demonstrate the *bacillus tuberculosis* in sputum, contrast staining should be employed. One of the small, cheesey-looking nodules which are always to be found in the fluid of tuberculous sputum, is with great care placed on a slide, then another slide is placed on the top, and the two gently rubbed together, so that the nodule is pressed out into an even layer. A single drop of distilled water is placed on a cover-glass, a small portion of the crushed nodule transferred to it with the aid of a platinum wire, and the material spread out over the cover-glass

as evenly as possible. When the prepared cover-glass is air dried, place it, bacteria-covered side downwards, on the surface of a little carbol-fuchsin in a watch-glass, and heat over a spirit lamp until the stain begins to steam, the cover-glass remaining in the warm stain for about two or three minutes. This will not only stain the tubercle bacilli, but the pus cells and any other bacteria that may be present. Place the cover-glass in a 5 per cent. solution of sulphuric acid for a few seconds, and then rinse in alcohol until no more colour comes away. Now transfer the cover-glass (still bacteria-covered, side downwards) to a watch-glass containing a watery solution of methylene blue, in which it should remain for eight or ten minutes; and then rinse well in water, dry, clear with oil of bergamot, and mount in xylol balsam. The slender tubercle bacilli will be found to stand out a deep-red colour, while any other bacteria and pus that may be present, are stained blue.

The vessel containing the infected sputum, and any apparatus that has been used,

must be thoroughly sterilised ere it is put away. The glass slips and vessel should be placed in a strong solution of carbolic acid and water and heated to 140° or 160° C. The platinum wire, directly the pus has been transferred on it from the glass slip to the cover-glass, must be made red-hot in the flame of the spirit lamp.

The novice should never lose an opportunity of preparing a slide of bacteria, and he will not have to seek far for material; sour milk or cream, rancid butter, tainted fish or meat, and the little nodules to be found on the roots of clover and peas, will all supply him with interesting forms.

Very beautifully prepared slides of typical bacteria are now to be obtained at a very moderate price, but the photo-micrographer when purchasing any of these slides, should be careful to select specimens which show good contrast, and have been stained deeply, choosing for preference those which have been prepared with a red stain.

CHAPTER III.

STEREO-PHOTO-MICROGRAPHY.

One of the most interesting and beautiful branches of photo-micrography, which as a rule receives little or no attention from the microscopist, is stereo-photo-micrography. This neglect is, without doubt, principally due to the fact that, owing to the wide and rapid spread of high-power microscopical research, the binocular microscope is now no longer used by the up-to-date scientist; but although the binocular is, of course, quite unsuited to any work requiring a higher magnification than from one hundred to one hundred and fifty diameters, the beauties of the stereoscopic image revealed by this medium-power magnification cannot possibly be realised by the student of to-day who works solely with the monocular instrument.

The non-possession of a binocular microscope, however, need by no means prevent the microscopist producing photographically some of the marvellous effects obtained by the aid of that instrument, stereo-photomicrography coming under the head of low-power work, the microscope itself is not required; and any one who possesses a half-plate camera, with a good extension of bellows, can, with the addition of two or three micro-objectives of moderate magnification such as a two-inch, one-inch, and half-inch, and a pair of "Stephenson's binocular prisms," successfully practise the art.

Stephenson prisms can be obtained from Messrs. R. & J. Beck, of 68, Cornhill; Messrs. Baker, of High Holborn, or any of the recognised makers of microscopes and microscopical accessories. They are frequently fitted to the compound microscope, when they are used as reversing prisms to facilitate dissecting operations, or the arranging of small objects, such as foraminifera, diatoms, sponge-spicules, etc., into groups or patterns. The prisms

are particularly useful to the photo-micrographer, as when they are employed there is no need to cut and transpose the

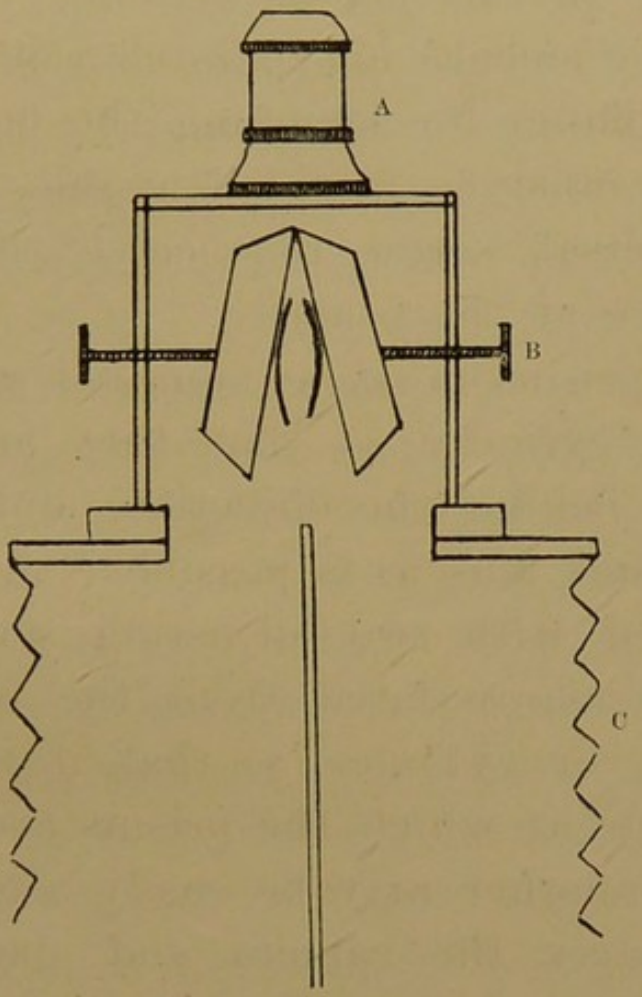


Diagram showing how the Stephenson Prisms are Mounted behind a Micro Objective for Stereo-Photo-Micrography.

A. Objective.
B. Stephenson Prisms mounted in a box or cylinder.
C. Camera with telescopic division in centre.

photographs, as is the case with ordinary stereoscopic work.

A short wooden cylinder should be pro-

cured to carry the prisms and objectives, or, failing this, a small well-made box will answer the purpose satisfactorily. The interior of the box or cylinder should be perfectly smooth and painted a dead black. A lens flange for attaching the box to the camera must be fastened at one end, and a universal screw to receive the micro-objective at the other.

The prisms must be mounted within the box or cylinder so that they are immediately behind the objective, and as near to its back lens as is possible; they should be fitted with two adjusting screws that can be manipulated from the outside of the box or cylinder, so that, if necessary, the angle at which the prisms are inclined to one another may be easily altered.

To carry the camera and specimen to be photographed, a base board, travelling stage, and object-holder should be constructed according to the directions given in Chapter I., and a telescopic partition must be made to run in an exactly central position through the entire length of the camera.

Stereo-photo-micrographic negatives will be found to require a slightly longer exposure than is necessary for ordinary low-power work, as the light has to pass through the Stephenson prisms before impinging on the sensitive surface of the plate. To obtain vigorous negatives, with good contrasts and full of detail, full exposure should always be given.

The best paper for making prints from stereo-photo-micrographic negatives is albumenised paper, or, failing this, Eastman's "Solio" P.O.P.; while the "Nikko" bromide paper made by the same firm, developed with amidol, and pressed into contact with a ferrotype plate to dry, will be found to yield most beautiful results by those who wish to be independent of sunlight, or who are only able to work during the winter evenings.

The most striking effects, however, are to be obtained by making transparencies from the stereo negatives, as it is possible, by using different developers, varying the length of exposure, and after-toning, to

obtain a very wide range of colour effects, from cold black to bluish-black, or different tones of brown and red. When finished and mounted, the prints and transparencies can be viewed with the aid of an ordinary stereoscope.

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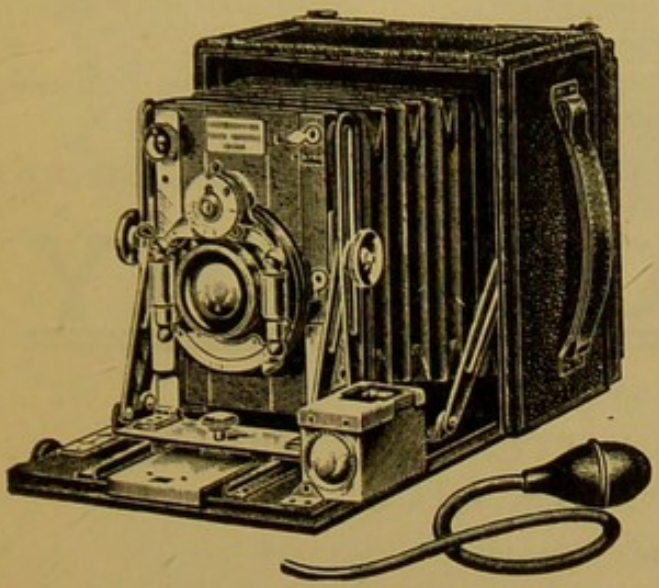

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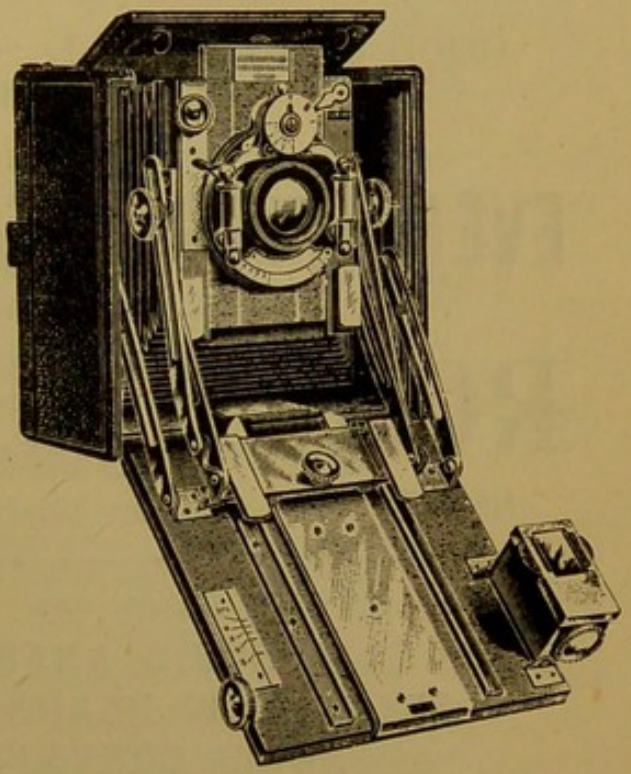

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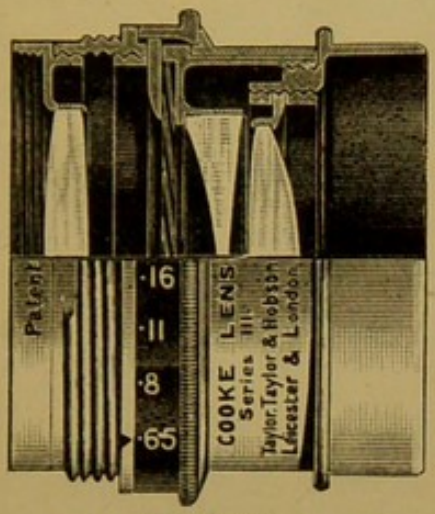

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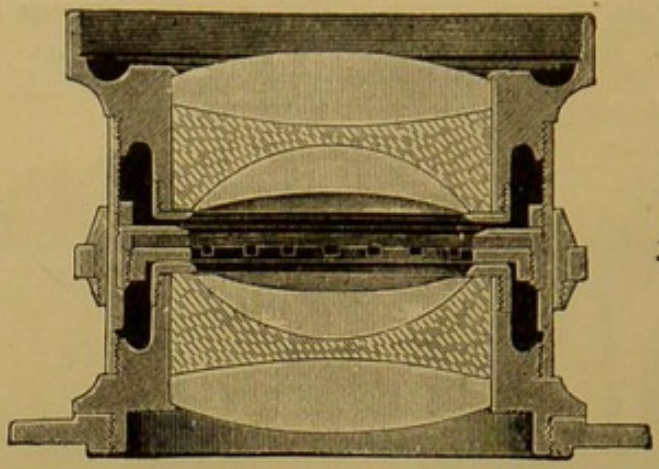

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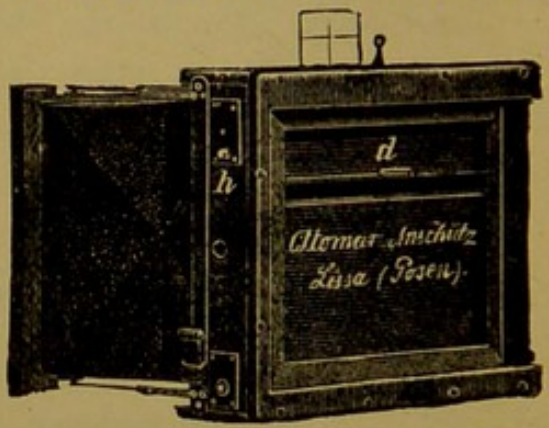

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