## JOURNAL

#### OF THE

# ROYAL MICROSCOPICAL SOCIETY;

### CONTAINING ITS TRANSACTIONS AND PROCEEDINGS,

AND A SUMMARY OF CURRENT RESEARCHES RELATING TO

ZOOLOGY AND BOTANY

(principally Invertebrata and Cryptogamia),

MICROSCOPY, &c.

Edited by

#### F. JEFFREY BELL, M.A.,

One of the Secretaries of the Society and Professor of Comparative Anatomy and Zoology in King's College;

WITH THE ASSISTANCE OF THE PUBLICATION COMMITTEE AND

A. W. BENNETT. M.A., B.Sc., F.L.S., Lecturer on Botany at St. Thomas's Hospital,
R. G. HEBB, M.A., M.D. (Cantab.), AND J. ARTHUR THOMSON, M.A., Lecturer on Zoology in the School of Medicine, Edinburgh,

FELLOWS OF THE SOCIETY.

#### FOR THE YEAR

1892.

Part 1.



PUBLISHED FOR THE SOCIETY BY WILLIAMS & NORGATE, LONDON AND EDINBURGH.

#### MICROSCOPY

#### a. Instruments, Accessories, &c.\*

#### (1) Stands.

The Binocular Microscope of the Seventeenth Century.<sup>†</sup>—Mr. Charles E. West writes:—"It is generally understood, I believe, that the binocular Microscope is a modern invention—that it goes no further back than the one made, about 1853, by Prof. J. L. Riddell, of the University of Louisiana. His instrument was a rude affair, made of two pieces of looking-glass, which was improved by Prof. A. K. Eaton, of this city, by substituting for the mirrors a solid prism of glass made of two triangular prisms which were cemented together. Riddell adopted the improvement. This is the basis of the modern binocular Microscope. Its perfection depends upon the equal division of the beam of light by the prism, or the uearness of that division into halves, so that the same amount of light may traverse each of the tubes to the eyes.

I never used but one instrument that did this, and that was made by J. Zentmayer, of Philadelphia. I tried it on the *P. angulatum* with the 1/5, 1/10, 1/15, 1/30, and 1/40 objectives, which resolved the frustule as satisfactorily as with a monocular. I have never found another binocular of this maker that would do this, and I have tried several.

But the first inventor of the binocular seems to have been Antonius Maria de Rheita, a Bohemian Capuchin, mathematician and astronomer, who published a work in 1645, under the title of 'Oeulus Enoch et Eliæ, sive Radius Siderio Mysticus,' a rare book, which I found in the Astor Library of New York.

By a contemporary writer, R. P. F. Ioannes Zahn, who published an optical treatise in 1685, entitled 'Oculus Artificialis Teledioptricus, sive Telescopium,' there is given a minute description of de Rheita's binocular Microscope and telescope. This work is in my possession, and from it I hope to prove that the binocular Microscope is no new thing.

In a letter to his friend, J. Caramuelis, dated Cologne, 24th April, 1643, de Rheita speaks of having detected most clearly, by means of his binocular telescope, 'with the greatest surprise, admiration, and delight, the sacred Sudarium Veronicæ sive faciem Domini maxima similitudine in astris expressum,' in the sign of Leo, between the equinoetial and zodiaeal circles. Zahn has given in his work a figure of our Lord's head as pietured on the handkerchief of St. Veronica. George F. Chambers, in his revision of Admiral Smyth's 'Cycle of Celestial Objects,' has given a reproduction of the figure, and characterized it a ' pious fraud,' A.D. 1643.

I propose to give a translation from the Latin of such parts of the 'Oculus' as have a bearing upon the binocular Microscope, as follows:-

'Since the distance between the eyes is not the same for all persons, first of all an artificer must ascertain this distance if he wishes to construct a tube perfectly fitting any one person. This can be best ascer-

\* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous.

+ Proc. Amer. Soc. Micr., xii. (1891) pp. 57-66.



н 2

tained (as elsewhere has been shown and as de Rheita also has said) by the aid of a pair of compasses and a mirror. This distance serves for adjusting the first ocular lenses, since in the binocular tube the two other lenses nearest the eye must be similarly placed to each other e. g. if the centres of the pupils of any person be distant A B, 22/100 pcd. Rom. (fig. 3, I.), at this distance also from each other must the other first lenses be, ab and cd, as is shown by the figure, so that the centres of the lenses ab and cd should meet at the points A and B.\*

'Let us suppose one simple tube be constructed with a convex ocular lens of very great aperture, which shall be greater than the distance of the two eyes, generally speaking it will be impossible, by using one convex ocular lens, however large, for an image radiating from the tube to affect both eyes alike, for if the image radiating from the tube through one lens could affect both eyes alike, both eyes must be at the point of contact; but this is impossible, since both eyes cannot be at the same point at the same time, and thus all parts and points of the image cannot be transmitted to the same parts and points of both eyes at once. I have said generally speaking, because if one look at a mirror of very obtuse convexity and at a great distance from the eyes, perhaps something can be effected: but it is of little practical use, for the objective lens must be of extremely long diameter to represent the object sufficiently large and near, so the tube would be obliged to be of enormous length.

'III. A binocular tube can be constructed with one objective lens when the image thrown from the objective lens can extend itself to a sufficient distance in the base of the divergence and thence again radiate through ocular lenses to both eyes. Thus the object C P D (fig. II.) radiating through the objective lens A B, forms the image E F; but the rays of the image, digressing from E, reach the eye N through the ocular lenses G I L, and from F reach the eye O through the lenses H K M. Thus a binocular tube can be constructed with one objective lens A B; but by this method the rays from the object reach the eye after being greatly refracted and diverging very far from the axis P Q of the objective lens, so that the image is never clear and distinct. Moreover, both eyes cannot see the whole of one object, nor is the same part of the object seen by both eyes, though more of the object can be seen by moving it about, but it will always be confused. Hence a tube thus constructed is never of much use.

'IV. Even if one large lens be taken having two apertures at exactly the proper distance apart, and both eyes look through these apertures at a single object, and thus see one and the same thing (which, however, seems impossible), nevertheless a perfect binocular tube cannot be formed, because, just as the radiation from the object comes to both eyes by rays remote from the axis, and hence more refracted, so will the image possessed in the cye be indistinct and confused.

'V. The best construction of the binocular tube is made by two telescopes exactly alike, so fitted to both eyes that the optical axis passes through one and the same object (figs. III., IV., VI., VII. VIII.). The

<sup>\*</sup> The plate is reduced one-half from peneil tracings. The letters on fig. I. were accidentally omitted. A B are the ends of the straight line, ab, bc the diameters of the circles on the same line prolonged. The circles represent the eye-lenses to be just as far apart as the observer's eyes.

two objective lenses must be exactly alike; not only of the same shape, but also alike in magnifying power and in point of elearness. (The same eare must be taken, whether the lenses be concave or convex.) Again, the telescopes must have the same "ordinate," so that not only the objective lines, but also the eyes similarly placed will correspond precisely to each other; but the telescopes and their first ocular lenses must be as far apart as is the distance between the centres of the pupils of the two eyes. The telescopes should be so directed toward one and the same object as if there were but one aperture for both tubes, and through this aperture the object, brought wonderfully near, can be very distinctly seen.

'VI. Since there are various kinds of telescopes, the ordinary Galilean, made of convexo-concave lenses, others convexo-convex, which can be made from pure convex lenses, and, indeed, again, out of these others can be so formed that they present objects inverted, as it is said to be the case with astronomical telescopes. Others present the object creet, but whether straight or curving inwards depends on the nature of the ordinate. The former can be made from four convex lenses, the latter from a plane glass and two very powerful lenses, &c. Thus also various binocular tubes can be made, depending on the ordinates of the telescope, provided that the ordinates be the same for the same binocular tube, taking the distance of the first ocular lens and the direction of both ordinates through one aperture, so to speak, so that the same object will be seen single and not double.

'VII. For lesser binoeular tubes common telescopes not exceeding a foot in length are best, because those more than a foot long do not present the object completely enough. The smaller the telescopes the greater the object. Tubes of this kind are made with three lenses for each telescope, having their ordinates placed as indicated in No. 13, eap. 5, seq. The proportion of the lines there indicated is also much approved.

<sup>6</sup> VIII. How to join the two telescopes. The two tubes may be made of copper or brass plates, as in fig. III., where two tubes A and B are joined by brass hinges at a b c; then, where the tubes are placed, both eyes are accommodated to any remote object, and the object will appear single and with great elearness. How the tubes may be joined, placed, elosed, and covered is shown in fig. IV. Likewise several movable rods similarly connected could be placed in each tube, but this method has disadvantages. Two tubes with their lenses arranged so as to be adapted to any vision are best constructed by cases or capsules as follows:— Make a capsule of brass leaf in the form of an ellipse, as shown in H (fig. VI.). Make two apertures in the lesser capsules E and F as far apart as the distance between the eyes. These apertures must also be elliptical, as E and F in the figure. The size of the apertures should be such that the tubes or rods A and B, also C and D, would perfectly correspond.

'The two dises E and F of both tubes must be so firmly fastened that the tubes will not slide up and down too easily. Thus we now have all things arranged as in the figure G. This entire affair ean be put into the greater capsule H and there encased. Thus it ean be easily and safely earried about. The eapsule H may be of wood, leather, or other material, and decorated to suit the taste.

'IX. Since the distance between the two eyes does not differ much

for many individuals, and also since the eyes easily accommodate themselves, many artificers fit their own eyes and fasten the rods in the tubes firmly in one position, so that they cannot be moved. I have made many such myself. Although they fit most people well enough, vet some eyes are unpleasantly affected after looking steadily for a long time. Least of all must such tubes be used for magnifying, unless they be adjusted to the distance between the eyes, since they harm the eyes not a little, and when used too much may even turn the eyes from their natural position (cross-eyed), as I have known happened to a certain nobleman. Thus it is always better to make the rods movable, so that they can be fitted to all eyes; yet it is allowable to make a little common binocular tube with movable rods, to be placed in a case after the manner of a little book, thus: Make a small capsule the size of the tubes which it is to contain, cover this with leather, and put ou clasps just like a little book. Put two thin wooden tablets at the ends where the ocular bands and objectives meet, but where the clasps are opened. These must be shoved aside, so that the eyes can look through the tubes. The cases may be so constructed that the open space between the two tubes can hold the "ignitabulum" with its sulphur thread (match-box) and the burning-glass for quickly making a fire in the field or anywhere you please. Above this space a cover of thin brass is placed, ou which is fixed a magnetic needle. At the other end, which is covered with leather where the book closes, can be placed a movable circle to point to the moon, according to the hour of the night; and thus various other things can be added.

'X. The long rods holding the lenses can be of various materials and shapes, but it is best to be so made that the lenses can be cleaned when necessary, for they will become dusty, no matter how carefully they are closed. That part of the tubes just before and just behind each ocular or objective lens should be so arranged that a small linen cloth could be inserted through an aperture for the purpose of wiping the lens.

'XI. In convexo-convex binocular tubes it is better for the first ocular lenses to be quite acute, so that the eyes can be placed nearer the same objects. They should always be a little more acute at the bottom than in simple convexo-convex tubes, since there is a greater clearness from the two eyes looking at the same thing. I have found these to be good proportions :- The objective lenses remove the focus to a distance of 11 ft.; the middle ocular lens, equally convex on each side, has a diameter 35/100 Rom. ft.; the first ocular lenses anywhere from a diameter 20/100. This is also a good binocular tube. Objective lenses remove the focus to a distance of 2 ft.; middle lenses, equally convex, 40/100 diameter; first lenses, near the eye, 20/100 and 25/100 diameter. Another good tube, objective lenses at a focus of 4 ft. Middle lenses, equally convex on all sides, 50/100 diameter. First ocular lenses, unequally convex, from diameter 30/100 and 35/100.

'XII. Two convexo-convex telescopes inclosed in a case can be made adjustable to any vision in the following manner: - Join the two telescopes by movable arms (as indicated in No. 8 of this chapter), and through these arms place a spiral screw, which can lengthen or shorten the telescope at will. This is shown in the 7th figure. A BC is a nail cylindrically round from A to B, spirally round from B to C. At B is a small nail, by which the large nail A BC is kept in place after being

102

place 1 through the round aperture D. Thus the arms G H and I H, joining the two telescopes, can be contracted or extended. The form E F is placed upon H, and both arms G H I are held in place by a small nail passing through F and H. There the spiral part B C is placed through E. Now, if the head A of the nail A B C be turned this way or that, the arms G H and I H, joining the two telescopes, will be contracted or lengthened.

<sup>c</sup>XIII. Cases to contain long binocular tubes should be made of strong solid wood and the tubes within so firmly made that they cannot bend; also that the glasses may at any time be taken out and the tubes differently placed, so as to be adapted to one vision or another. The upper part of the case must be so fitted with clasps that it can be closed or opened at pleasure. Long tubes of this kind can be made square like oblong beams, and may be made of plates of alloy of silver, lead, and iron, joined in several pieces, which can be easily separated from each other again. I saw a tube of this kind constructed by P. Rheita. It was at the Castle of Herbipolensus.

'XIV. The longer the binocular the better, but the less convenient. How the inconvenience may be remedied I will show. Where these tubes are to be used they may be placed on long poles and easily extended or contracted. Tubes of this kind are very useful in war for viewing the enemy from afar, &c. I have selected the construction of a tube of this kind which I have heard P. Rheita used in his wonderful binocular telescope, whose lenses were not fitted to tubes, but to a certain capsule which could be folded like a pair of bellows. The length of this was about ten hands when extended on a polc. They say that it made the moon of enormous size, which can easily be believed. The manner of constructing this kind of binocular tube is as follows :- In the first place, make tubes out of leather folded like paper lanterns, or like the leather pipes used by hunters and bird-catchers for alluring beasts and birds. Crumpled leather tubes of this kind can be fitted to transverse plates, in which are placed the glasses, as in A B C D E F, fig. VIII. These plates vary in number according to the length of the tube and hold the tubes perfectly straight, and also (since they have holds within) transmit to the eye a clear image, as is the case commonly in other telescopes. The transverse tablet is shown in G H in the figure. Again, that access may be had to the ocular glasses, some part of the transverse plate B and C can protrude on either side (as is seen in J K). to which are attached the leather tubes after the manner of a capsule. When the tubes are to be attached to the pole for use, cords or little chains a b c d e are put through all the transverse tablets, and at each of these tablets a knot is made, or in some other way the exact length of the desired extension is kept. Then nails at a and f fasten the tube to the pole and hold it at the desired extension. It can be constructed as in M and inclosed in the capsulo N (for it must not be kept stretched on the polc all the time), and thus conveniently carried about. Instead of a pole, a contrivance like that shown in V P can be used, which can be extended to any length and again folded together, as in fig. 2, and easily carried about ; also one large leather tube holding both telescopes can be made, which can be extended at pleasure like a pair of bellows. Many of the contrivances might be mentioned for showing the object right side up in astronomical binocular tubes, &c.'

Binocular Microscope.-Take for the objective glass a lens equally convex on all sides, of a diameter 80/100. The principal focus will be at a distance 40/100. Now remove the object a little beyond this distance, so that the rays behind the objective lens can converge to form an image. This image will be much larger than the object (as we have shown in formula 2), for at double the distance it will be equal to the object itself, and from double the distance till you reach the focus it (the image) will always be larger, till at the focus there will be no image at all, because there the rays behind the lens are parallel. Tho object placed beyond double the distance of the focus always makes the image less and less than the object, till it is placed at so great a distance that rays falling upon the lens are considered parallel and the image is found in the distance of the principal focus. All this has been already shown elsewhere. Behind this greater image two other ocular lenses are placed. One near the image itself is exceedingly convex on all sides, of a diameter 25/100. This arrangement shows the object very large



and a little farther away than in ordinary composite Microscopes shown in cap. 2.

On account of lengthening the tube, or the greater distance of the lenses from cach other (if a similar arrangement be made for both eyes), both cyes can easily look at one and the same object. The two 'prios' lenses, ocular lenses, should be as far apart as is the distance between the two eyes, and all else arranged as in binocular tubes already explained. Such a Microscope in its external form may be like the one shown in the figure, where A B is the case. Within are the two tubes, arranged as we have already shown in treating of binoculars. The object to be looked at is placed at C."

Binocular Perimicroscope.\*—This instrument, devised by Aubert, is practically Westien's binocular lens with much increased magnifying \* Pflüger's Archiv, xlvii. (1890) pp. 341-6 (2 figs.).

power. As the accompanying figures (4 and 5) show, the instrument consists of a double tube, supported on a stand, consisting of a base-board A and two vertical pillars B B'. The objectives are so adapted that the lines of vision unite exactly on the object. The true field of vision has, with a magnification of 25 diameters, a diameter of 10 mm., the distance between the objective and the object being 40 mm. The double tubes alter their position in a vertical direction by means of the cylinders L,



which work in the tubes G, and they are balanced by the weight O. Horizontal and transverse movements are imparted through the cylinders N N', which are connected with the pieces K K'. Owing to this mobility, an object of  $17 \times 9.5$  cm. = 161.5 qcm. can be examined, hence the name Perimicroscope. The instrument is adapted to the examiner's eyes, or rather, the distance between two pupils, by pulling out the oculars until a single image is seen. The instrument is focused with one hand, and owing to its mobility, obliquely lying surfaces can be examined. The object to be examined is placed on a black, white, or green glass plate placed on the stand A, which will, of course, receive a small vessel or any other object-holder.

Lantern Microscopy.\*—The following correspondence has appeared on this subject:—"Permit me to say a few words in connection with \* English Mechanic, liv. (1891) pp. 309-10, 332. Mr. Chadwick's paper on Leach's Lantern Microscope, reported in the issue of Nov. 13th.

In the first place, Mr. Chadwick is dead against every authority regarding magnification. Extreme magnification, such as Mr. Chadwick suggests, is condemned by every authority of note, both for the Microscope and for the lantern Microseope. That the magnification which enables the observer to see all the details of an object is the largest to which it should be subjected, anything beyond this defeating the purpose. Mr. Chadwick says that, with his arrangement, high powers can be used for class and other demonstrations, although he has previously stated that the arrangement of condensers is only adapted for objectives of 4/10 in., which is by no means a high power. Mr. Chadwick eannot surely be ignorant of the fact that lantern Microscopes, of whatever make, have long been discarded by college authorities where *high* powers are required. I see, too, that he has come round to my views, and does not recommend the use of special objectives.

His last paragraph but two is a contradiction of the previous portion of his paper, where he leads us to suppose that he can use high powers—that is, of course, 1/15 in. or 1/20 in. objectives—with his arrangement, simply because he uses an alum-trough. What he says is simply this: in order to use a high power requiring a large amount of light, interpose an alum-trough which stops 50 per cent of the light. Mr. Chadwick does not seem to be aware of the fact that it is possible to focus the heat-rays at a point beyond the luminous rays, and thus avoid all heating of the slide. I should just like to inform Mr. Chadwick that the substage condenser of which he speaks, and the novel way of interchanging the objectives was used by me more than sixteen years ago, and shown to Mr. Leach in 1876.

In conclusion, Mr. Chadwick says that lantern Microscopes which have no alum-trough are self-condemned. This I deny, as I do not use an alum-trough and never will, and I may say that I have never melted a slide.—J. A. FURNIVEL."

"I am pleased to see Mr. Furnivel's plain and simple defence of himself on this subject. He may fairly be considered the father of the simple lantern Microscope, and as I have worked with both his simple form, without alum-trough and also with Leach's, I may say that experience fully bears out his statements. That it is possible with either form to get a large projection on the screen with a good limelight is no doubt correct; but after a most careful testing of both under precisely the same conditions, I most certainly prefer Furnivel's at less than half the price. My object in testing both was to obtain a clear definition of the different yeast-cells on the sereen, but after repeated trials, with the assistance of some experts with the lantern and Mieroscope, we failed totally with both, the best results being obtained with Furnivel's arrangement. With a Powell and Lealand 1/4 in apochromatic the results under the best conditions were most unsatisfactory, and we found with either form the highest power which could be used was a 4/10.

It sounds very large indeed to talk about the probose of a blow-fly 16 ft. long; but the fact is that the same would be far better shown if only 3 ft., and would make a much more effective picture in any ordinary

106

room. I have no experience with the arc light in the micro-lantern, and therefore my remarks do not apply to this .- THOS. FLETCHER."

#### (2) Eye-pieces and Objectives.

Zeiss's New Microscope Objective.\*-Count F. Castracane referred to the new Zeiss's objective of 1/10 in. focal length and numerical aperture 1.63, and the beautiful photomicrograms of Amphipleura pellucida taken by Dr. Van Heurek to test its value. In these the square structure of the minutest grain is clearly visible. The magnification employed amounted, according to Van Heurck, to 2000 diameters, and consequently the longitudinal divisions would amount to 5000 to the mm., thus exceeding considerably the limit of visibility determined by Helmholtz's theory. As no mention was made of such a result by Prof. Abbe when he demonstrated the utility of an illuminating conc of wide aperture in connection with the new Zeiss objective, it seems possible that Van Heurek has made a mistake in assigning the magnification, especially when it is considered how little reliance can be placed upon the magnifications given by the makers of Microscopes.

#### (3) Illuminating and other Apparatus.

New Hot Stage.<sup>†</sup>-M. Drosten describes a new hot stage which will be found very serviceable in microscopy. It consists (fig. 6)

of a shallow box made of glass plates soldered together by an enamel which is not affected by heat or liquids. In one of the side walls of the box a thermometer and two glass tubes a and b are fixed. The heating of the stage is

effected by a stream of warm water, which enters by the tube a and passes out by tube b. By regulating the flow of water in b by means of a clamp a very constant temperature, only varying by half a degree, can be obtained. The stage, being entirely of glass, can be used with all objectives up to the highest magnification.



Glass Slide-boxes. ‡-M. Drosten describes slide-boxes constructed of glass plates united by an enamel which is not dissolved by alcohol

\* Atti Acc. Pontif. de' Nuovi Lincei, xliii. (1890) pp. 215-6. † Bull. Soc. Belg. Micr., xviii. (1891) pp. 5-7. 1 Ibid.

F1G. 6.  or any other liquid. The large model (fig. 7) is provided with grooves for the reception of slides of  $76 \times 26$  mm., and the small model is intended for cover-glasses of 18 mm. in the side.

Arsonval's Thermostat modified for Benzin-heating.\*—Mr. J. Ogmannikow has so modified Arsonval's gas thermostat that it ean now be heated with a benzin lamp of special construction, for the details of which the original must be consulted.

The temperature maintained is said not to vary more than from  $0^{\circ} \cdot 1$  $-0^{\circ} \cdot 4$  and usually not more than  $0^{\circ} \cdot 2$ . Where gas is not obtainable a device of this sort is very useful.

#### (4) Photomicrography.

**Photomicrography.**<sup>†</sup> — For photomicrographical work the two requisites are a Microseope and a eamera. The Microseope must possess an arrangement which allows the body-tube to be brought into a horizontal position while the foot rests firmly upon the table. The eamera differs from the ordinary portrait eamera in the greater length to which it ean be drawn out. Such apparatus have been supplied for years by very many firms; but they are either very expensive or, if eheap, are very defective, not possessing generally the length of bag which is indispensably necessary for taking bacteria, diatoms, &c. The institute of Klönne and Müller, of Berlin, has lately supplied a cheap and at the same time neatly constructed apparatus which answers every require-Dr. R. Neuhaus, an authority on photomierographical work, ment. describes this apparatus in his 'Anleitung zur Mikrophotographie.' The camera can be drawn out to 1.80 m., and as there is light-proof connection of this with the body-tube there is no direct contact of the two. Sunlight reflected from a heliostat is the most convenient source of light. As the best substitute for sunlight, Neuhaus proposes the electric are light, while other authors regarding this as too unsteady, recommend the magnesium, or still better the zircon light. But petroleum light is sufficient for almost all purposes. The proper method of illumination is by means of the condensing system to form an image of the source of light in the preparation. Most photomicrograms have been hitherto obtained by the use of the Mieroseope objective without the eye-piece; lately, however, very well made projection eye-pieces have been recommended. The best safeguard from any shaking of the instrument during the exposure is afforded by a triple layer of thick felt under each leg of the table on which the apparatus stands. No preeise directions can be given as to the time of exposure necessary for the production of a good negative. By direct sunlight, with the strongest magnifications and sufficient weakening of the light by the filter, a few seconds are sufficient.

On the value of photomierography the most contradictory opinions are held. Some think that in a short time it will quite drive drawing from the field, while others, taught by sad experience, are inclined to depreciate its importance. Both are wrong. Certain things will always remain to the draughtsman, while others belong exclusively to the photographer.

<sup>\*</sup> Wracz, 1890, pp. 725-6. See Centralbl. f. B.kteriol. u. Parasitenk., x. (1891) p. 132. † Central-Ztg. f. Optik u. Mechanik, xu. (1891) pp. 262-3.

For photographic purposes, only thin preparations which lie as much as possible in one plane are suitable. For this reason diatoms have been the chosen subject of representation. Only the thinnest sections, as obtained by the most perfect microtomes, are suitable for photography. Of thicker preparations excellent drawings can be made. In not a few cases a certain thickness of the object is absolutely necessary, viz. when the essential details naturally lie in different planes. The draughtsman, by constant turning of the micrometer screw, combines the different planes into one; in photography such a process is impracticable. The discredit which has fallen upon photomicrography is for the most part to be ascribed to the ignorance and want of skill of those who would be always photographing objects which are absolutely unsuitable for photographic purposes.

#### (5) Microscopical Optics and Manipulation.

The Measurement of Lenses.<sup>\*</sup>—The following is the valuable cssay recently read by Prof. Silvanus P. Thompson to the Society of Arts:— Often have I regretted that the resources at the disposal of the Technical College, Finsbury, did not enable its staff to organize and equip a proper laboratory for optical measurements, and for the standardizing of optical instruments, in the same thorough and practical way in which they have now for more than ten years organized laboratories for electrical measurements. What Prof. Ayrton did ten years back for tho City and Guilds Institute, in organizing a laboratory for electrical measurements, I have longed to do for optical measurement, believing that, when the opportunity should come, the work would be of as much benefit to the optical industries of London as the electrical laboratories of the City and Guilds Institute have been.

The exact measurement of optical quantities is no novelty: for in this branch of science precision has long reigned, if not in the lower branches of the industry, at least in the higher. And the laboratory methods of optical measurement are for the most part thoroughly worked out and known, though many of them unfortunately involve the use of expensive instruments and appliances.

An optical laboratory should possess the means of testing rapidly, accurately, and without too expensive appliances, such matters as the truth of plane surfaces, the curvature of curved ones, the focal powers of lenses, their aberrations and their aperture. It should have means of testing mirrors and prisms, as well as actual entire instruments. It should be able to state the results in terms available for future years by the employment of accurate fundamental standards.

With but one very small part of the subject of the work of the optical laboratory do I propose to deal to-night, namely, with the measurement of lenses. Lenses are used for many different purposes, and in varied functions and combinations. Measurements that would be important for some of these are utterly unimportant for others. For example, the condenser lenses used for magic lanterns are not wanted to be either aplanatic, achromatic, or rectilinear; their function being merely to collect the light which emanates from a certain luminous

\* Journ. Soc. Arts, xl. (1891) pp. 22-39. Reprinted by the author's permission, and with his corrections.

patch, and spread it as nearly equally as possible over the area covered by the transparent slide, so that the whole is equally illuminated, and so that the light so transmitted shall be on the whole slightly convergent. To measure the aberrations or exact focal powers of such lenses would be a useless work.

However, it will be convenient at the outset to enumerate all the things which might be made the subject of measurement with respect to a lens or combination of lenses. These are no fewer than eighteen in number:—(1) Diameter, or linear aperture. (2) Thickness, or length from pole to pole. (3) Focal power, or its reciprocal the focal length. (4) Position of principal focal planes. (5) Position of optical centres ("principal points" of Gauss). (6) Angular aperture. (7) Chromatic aberration. (8) Spherical aberrations, lateral and longitudinal. (9) Chromatic difference of the spherical aberration. (10) Loss of light by reflexion from surfaces. (11) Absorption of light in transmission. (12) Illumination of field, central and marginal. (13) Complaneity of focus (included in 7 and 8). (14) Degree of distortion of image (rectilinearity). (15) Cylindricity, or degree of astigmatism, including angle of axis of cylindricity. (16) Accuracy of centering. (17) Definition in margin of field (involved in 7, 8, and 16). (18) Refractive indices of materials.

Now, of all these varied matters, there are but three with which the present paper will deal: namely, the focal power of lenses, and the position of their focal planes and principal points.

By focal power I mean, of course, that property on which their convergivity (positive or negative) depends, and on which in turn their magnifying (or minifying) action is dependent. It must be borne in mind, as a fundamental principle of elementary optics, that all that any lens or mirror (or combination of mirrors or lenses) can effect is to imprint a curvature on the wave-front of the light that enters it. If the wave is plane—i. e. consists of parallel rays, to use the old language—then the lens prints a curvature, positive or negative, upon it by virtue of which its march is changed, and made convergent or divergent. If the wave before impinging on the lens is initially non-plane, but either convergent or divergent, then the lens will alter the curvature of the surface, the resultant curvature on emerging being simply the algebraic sum of the initial curvature and the impressed curvature.\*

The focal power is the curvature imprinted by the lens on a plane wave, and is the reciprocal of the true focal length. It is appropriately expressed in terms of the proper unit of focal curvature, the *dioptrie*.<sup>†</sup>

\* All the ordinary formulæ of the text-books for lenses are more or less particular statements in symbols of this general rule; for example, the well-known formula

$$\frac{1}{v} = \frac{1}{u} + \frac{1}{f},$$

in which f is the focal length, and u and v the respective distances of two conjugate points serving as object and image. The reciprocal of a length *is* a curvature; so that this formula merely states that one curvature is the result of adding two other curvatures together. I have pointed this out in a paper in the 'Philosophical Magazine,' in Oct. 1889.

<sup>†</sup> The *dioptrie*, originally proposed by Monoyer as the unit of focal power of a lens, is now in international use, having been formally adopted by the International Medical Congress of Brussels. It is a unit of curvature, and as such may be used The proper numbering of lenses in dioptries has been an enormous gain in one branch of the optical industries—that of the ophthalmists—and it is much to be wished that other lenses than those of spectacles should also be henceforth described in the same way.

Another important step has been the introduction of the conception of focal planes-a conception to which the use of the photographic camera has doubtless contributed its share. Less fully recognized, but none the less important, is the conception introduced by Gauss as the result of his studies in geometrical optics, that the properties of any centered combination of lenses might be represented by a system of planes and points which are, so to speak, the characteristics of the equivalent lens. A lens can be considered as having a single optical centre only when it is infinitely thin, or at least of negligible thickness. All thick lenses and combinations of lenses have two optical centres, described by Gauss as the two "principal" points ("Hauptpunkte"), which are considered as the places where the axis intersects two " principal planes." These principal planes are at a certain distance apart, and equidistant between the two principal foci at the back and front of the lens. They possess certain properties most useful in the geometrical treatment of lens problems, and act as though the light, however obliquely it may be crossing the lens, were transferred straight from one to the other. The two "principal" points, or optical centres, possess

the property that light proceeding from any direction towards the one of these points passes out from tho lens combination as though it had passed through the other. Fig. 8 shows a thick lens (in diagram), in which the two principal points of Gauss are marked  $H_1$  and  $H_2$ , with the two principal planes drawn through them. These two points, together with the two principal foci,



completely determine the action of the lens. When the positions of these four \* cardinal points are known for any lens or lens-system, then all is known that is necessary for a complete discussion of the formation of images for all objects lying near the principal axis. The true focal length is the distance from either of the two principal planes to the corresponding principal focus; the back focus and the front focus being

for other purposes than focal powers—for example, to express the curvatures of surfaces. Unit curvature is taken as the curvature of a circle of one metre radius. Or a lens having a metre focal length is described in modern language as having a power of one dioptrie. A lens having half a metre as focal length has a power of two dioptries.

<sup>\*</sup> If the lens system is not bounded by similar media on its two faces—for example, if one side is bounded by air and the other by water—the two optical centres are shifted away from the two principal planes towards the denser medium, and are known as "nodal" points. In this case there are six cardinal points to consider.

equidistant each from its own optical centre. But hitherto it has been found difficult to ascertain the position of the principal planes of a lens combination. Optical instrument makers generally have no information on the subject that they can furnish. They can tell us approximately the focal length, but they cannot, or do not, tell us the position of the optical centres or principal points from which this focal length is to be reckoned. Beginners in microscopic work, when they come upon an objective marked "1/4-inch," expect to find that the object must be placed 1/4 in. below the front surface of the lens, and are often puzzled to find that the distance is perhaps only 1/10 in. Naturally they ask the question, from what point does the maker measure his quarter of an inch? The correct answer is that the 1/4 in. should be measured from the principal plane which corresponds to the front focus of the lens. But where this principal plane lies is never marked on the brass mount of the lens, though it ought to be. Again, the beginner often asks what is the correct way to reckon the true tube-length of the Microscope between objective and eye-piece? Must he measure from the lowest point of the eye-piece to the highest point of the objective, or how? The right answer is that the true distance between objective and eyepiece is not the mere length of the tube, but is the distance between the second principal plane of the objective, and the first principal plane of the eye-piece. But how is the unfortunate possesser of the instrument to measure this if the constructor has omitted to mark on the eye-pieces and objectives the positions of the principal planes?

It is one of the purposes of this paper to describe an instrument for measuring lenses, and ascertaining the precise position of these principal planes. Therefore, a few more preliminary words about the principal planes and the two Gauss-points through which they pass, will be appropriate.

In ordinary single lenses, if not very thick in proportion to diameter, the distance between the two principal planes is approximately onethird of the thickness of the lens at its middle. Exact formulæ are given in various modern treatises on geometrical optics. In lenses that have their two faces of different curvatures the principal planes do not lie symmetrically between the two poles\* of the lens, but are shifted toward the more highly curved face, or even beyond it. In plano lenses,



whether convex or concave, one of the two principal planes is a tangent to the curved face. These matters are illustrated by the sketches in figs. 9, 10, and 11.

\* I have used this term for years to denote the middle-points of the two faces of the lens, and find it very convenient.

If the positions of the four cardinal points are known for any two lenses separately, then, when the lenses are placed at any given distance apart, the positions can be found for the four cardinal points of the combination. The geometrical construction is very simple, and is illustrated in fig. 12. Let  $h_1 h_2$  be the two principal points of a lens,



and f its principal focus for light passing through it to the right. Let  $h'_1 h'_2$  be those of a second lons, and let f' be its principal focus for light passing the other way. It is required to find the position of the principal points and of the principal focus of the equivalent lens. Consider any ray-path a b parallel to the axis. Light travelling along from a will, after passing the principal planes of the first lens, turn towards f. Similarly, light passing the other way from b, after passing the second lens, will turn towards f. These paths cross at o. Join o  $h_2$ and  $o h_1$ ; and draw  $h_1 x$  parallel to  $o h_2$ ; and  $h'_1 y$  parallel to  $o h'_1$ . The planes  $x H_1$  and  $y H_2$  drawn through x and y will be the desired principal planes. And the resultant focus F is found by considering the ray which starts from a and passes through o towards f, and remembering that, as it passes through the second lens, it will be shifted forward through the distance between the planes  $h'_1 h'_2$ , and turned as though it camo from y. A little consideration will show that if the two lenses were close together the width  $H_1 H_2$  will be the sum of the widths  $h_1 h_2$ and  $h'_1 h'_2$ ; whilst if the two lenses are moved wider apart H<sub>1</sub> and H<sub>2</sub> will come nearcr together, and may even cross past one another. If tho lenses are placed at a distance apart equal to the sum of their focal lengths,  $H_1$  and  $H_2$  will not only have crossed planes, but will have separated to an infinite distance apart.

The formulæ for calculating the resultant focal length and resultant width between the principal points for a combination of two lenses at a distance apart, are as follows :—

resultant 
$$f = \frac{f_1 f_2}{f + f_2 - a}$$
;  
resultant  $w = w_1 + w_2 - \frac{a_2}{f_1 + f_2 - a}$ ;

Ţ

1892.

where a is the distance between the lenses,  $f_1$  and  $f_2$  their focal lengths,  $w_1$  and  $w_2$  the widths between their principal points.

From these formulæ it follows that the resultant focal length of a combination of two positive lenses when separated by an interval is always greater than the reciprocal of the sum of their reciprocals, and increases as the lenses are separated. The resultant width between the principal points decreases from the value  $w_1 + w_2$  down to nothing, and then increases negatively, becoming infinite when  $a = f_1 + f_2$ , and again becoming positive and finite when a exceeds that value.

If the optical combination is not achromatic, then the positions both of principal planes and of principal foci will be, in general, different for lights of different colours.

There exists yet another pair of points and planes, having special properties that should be noted. These are the points situated on the axis beyond the principal foci at distances respectively equal to the true focal length on either side. They are marked  $S_1$  and  $S_2$  in fig. 13,



being conjugate and symmetrically situated. Toepler, who first called attention to these two points, called them by the not very apt name of negative principal points (negative Hauptpunkte). I call them the two symmetric points, and the planes through

them the symmetric planes. They possess the very useful properties that any object in one has an image of equal size, inverted, in the other, and that any ray which crosses one of them at any distance from the axis will, after traversing the lens, cross the other symmetric plane at an exactly equal distance from the axis, but on the other side of the latter.

I have now cleared the way for discussing the methods that have been hitherto used or suggested for measuring the focal properties of lenses. Unfortunately, most of the ordinary methods of focometry which are in accepted use are based on the assumption that the lens may be treated as of negligible thickness, and in some others one has to make assumptions beforehand as to the probable width between the principal planes. This is easy enough in the case of a single lens, but for compound lenses the principal planes come in most unexpected positions, at an unknown width apart. After I have enumerated the methods of focometry, and briefly described each, I will describe a new method, and an instrument for carrying it out.

#### METHODS OF FOCOMETRY.

The methods of focometry may be classified under six general heads; and under these may be grouped the several varieties adopted by different authorities.

I. Methods of Direct Focal Scarch.—(a) Objective Methods.—The classic simple method of ascertaining the principal focus of a lens or optical combination, applicable only to positive lenses; consisting in sending rays from any very distant object through the lens, and search-

ing for the real image which is received upon a suitable surface, preferably that of a semi-transparent screen.

(b) Berger's \* Method.—A variety of the preceding, in which an illuminated object and a collimating telescope at a finite distance are substituted for an indefinitely distant object, and the real image at the principal focus is sought by means of an observing Microscope.

(c) Maskelyne's † Method.-A telescope, fitted with crosswire eyepiece, is focused upon an object at indefinite distance. The (positive) lens under examination being then clamped over the object-glass, it is pointed to a near object, which is moved to such a position as will give an accurate image in the telescope, the position of the object being that of the principal focus of the lens.

(d) Merz's ‡ Method.—A variety of the preceding, adapted to negative lenses. The telescope, with the lens under examination fitted in front of the object-glass, is focused upon an object at an indefinite distance, and then the lens being removed, is pointed to a near object which is moved to a position giving accurate focus.

(e) Kerber's § Method.-The positive lens being placed in front of an illuminated slit, it is moved to such a position that a slab of glass with parallel faces introduced into the path of the emerged rays produces, on being tilted, no change in the apparent position of the slit as viewed in a telescope; this being a test of parallelism of the rays.

None of the foregoing methods are applicable to very short focus lenses, and they give no information as to the Gauss points.

(f) Pendlebury's Method.—The positions of each of the two principal foci are found objectively, and then the respective distances are found from these to two conjugate points by inserting a luminous object and finding, objectively, its image. Then calling these two distances p and q, the true focal length is found by Newton's rule as a geometrical mean between them. After this calculation has been made, the distance between the two principal points can be found by subtracting twice the true focal length from the distance between the two principal foci.

II.—Methods of Magnification.—(g) Ramsden's Method.—Measurcment is made of the size and distance of the real image formed at the conjugate focus of an object of known size, at known distance: and from these the focal depth is calculated. This method assumes the lens to be of negligible thickness.

(h) Meyerstein's ¶ Method.—This is a modification of Ramsdon's

\* Berger, 'Apparat zur genauer Bestimmung der Brennweite von Objectivgläsern,' Zeitschr. f. Instrumentenk., vi. (1886) p. 272.

After considerable scarch I am unable to find any writing of Maskelyne's which describes the method which goes by his name. The same remark applies to Ramsden's method.

t Merz, 'Ueber eineu neuen Apparat zum Messen der Brennweite,' Pogg. Aun., lxiv. (1845) p. 321. § Kerber, 'Verfahren zur Bestimmung der Brennweite von Linsen,' Zeitschr.

f. Instrumentenk., i. (1881) p. 67.
Pendlebury, 'Lenses and System of Lenses' (London, 1884), p. 82.
Meyerstein, 'Apparat zur Bestimmung der Brennweite sphärischer Linseu und

Linsensysteme,' Wied. Annalen, i. (1877) p. 315; and Carl's Repertorium, xiv. (1877) p. 363.

I 2

method to meet the ease of thick lenses. The lens is placed between an object and a screen fixed at more than four times the focal length apart. Measurement is made of the size of object and image, together with the distance of the lens from one of them. The lens is then reversed end for end, and is displaced longitudinally until the same magnification is obtained as before on the same screen. All assumptions about the Gauss points are thus eliminated, for it is elear that if in the second observation, after reversing the lens, the samo magnification is obtained as in the first, the second principal point now occupies the position which the first principal point previously occupied, and vice versa. From the magnification, the distance between object and image, and the measurements of the displacement of the lens, the true focal length is ealeulated. Dr. Meyerstein describes a special instrument for earrying out this method. This method was devised in 1844, but not published until 1877, after the same principle had been independently discovered and described by Dr. Hoppe.\*

(i) Hansen's † Method.-An object of given size being chosen, the positions of the lens are found which give (real) images of linear magnitudes respectively equal to that of the object multiplied by 1, 2, 3, &e.; and from two (or more) of such observations, each of which requires a series of double adjustings, the focal length and the distance between the principal points are calculated.

(j) Mergier's ‡ Method.—This is an elegant mode of carrying out one of Hansen's suggestions. The two symmetric points are found by trial and double adjustment, as in Silbermann's method mentioned below, the magnification here being unity. Two micrometers serve as respecting object and as receptive surface for the image. This adjustment being made, then, in order to produce an image in the same place as previously but of double magnitude, it is sufficient to displace the lens through a distance equal to the foeal length, and the object though exactly half this distance. This is accomplished by simple mechanical means, with two serews connected by wheel-gearing.

III. Methods of Unit Magnification .- These methods constitute a special case of II., but are quite distinctive.

(k) Silbermann's § Method.—In this method the lens (positive) is placed at the middle of a graduated bench, upon which two transparent micrometers are placed on either side, so that the image of one micrometer falls upon the other. By a well-known theorem, the total distance between object and image will be a minimum, when the distance between them is equal to four times the focal length, and each is situated at one of the symmetric points of the lens. The

\* Hoppe, 'Ueber die Bestimmung der Haupt- und Brennpunkte eines Linsen-

systems,' Pogg. Annalen, elx. (1876) p. 169. † Hansen, 'Untersuchung des Weges eines Lichtstrahls durch eine beliebige Anzahl von brechenden sphärischen Oberflächen,' K. Sächs. Gesellsch. f. Wissenschaften, xv. (1871).

t Mergier, 'Nouveau focomètro pour la détermination des constantes optiques des systèmes dioptriques en général,' Séances de la Société de Physique, 1887, p. 193.

§ Silbermann, Comptes Rendus, xiv. (1830) Feb. 22, p. 340. See also Verdet, <sup>4</sup> Cours de Physique,' wherein it states that this instrument was referred to a Com-mission, consisting of MM. Arago, Babinet, Biot, and Pouillet. No reference to any report of this Commission can be found.

operation of finding these symmetric points consists, then, in a series of double adjustments of the following kind :- One of the micrometers being placed at a distance judged approximately as twice the focal length, the second micrometer is then moved, until upon its surface an exact image of the first is formed. If, on comparing the size of the divisions of this image with those of the surface on which it falls, it is found that they do not coincide, but are either magnified or minified, the distance of the first micrometer is either increased or diminished. and the second micrometer is again adjusted to the new position of the image, and a fresh comparison made. By successive trials and approximations the symmetric points are approached; and, when so found, the distance between them is measured, and one-fourth of it taken as the focal length. The method is open to the objections (1) that it is inapplicable to thick lenses, as it does not take into account the distance between the Gauss points, and (2) that it requires a tiresome series of The simple modification of this method, suggested double adjustments. by Webb,\* needs no notice here.

(1) Donders'† Method.—Donders improved the method of unit magnification by substituting for the series of double adjustments a simpler mode of equalizing the size of object and image. He used as object an opaque screen provided with perforations, the linear dimensions of which from one to another were measured with a Helmholtz's ophthalmometer. The lens to be measured being placed in front of this object, an image is then formed on a translucent screen, and the lens is then moved to increasing distances until the size of the image, as measured by the ophthalmometer, is equal to that of the object.

(m) Snellen's ‡ Method.—This method, which closely resembles that of Donders, is carried out by the aid of an instrument called a *Phakometer*, consisting of a graduated bench at the middle of which the lens is placed. No ophthalmometer is used to measure the sizes of object and image, a translucent screen figured with marks serving to detect any want of equality between the sizes of image and object. It is assumed that the lens has a single optical centre at its centre of figure, and a mechanical adjustment serves to movo the object and the screen at equal rates from the lens at the centre. The scale is divided to read off direct in *dioptries* the focal power of the lens. Snellen applies the method to negative lenses and long-focus positive lenses by placing them between two positive lenses of equal and known focal power; and in the case of plano-convex and meniscus lenses, he recommends that to secure symmetry, such lenses should be taken in pairs, back to back, and measured together.

IV. Methods of Approximate Unit Magnification. — (n) Bessel's § Method.—In this method the object and the apparatus to receive the image are placed at a distance from one another, exceeding four times

<sup>\*</sup> Webb, 'Literary Gazette,' 1857, p. 1101; and Fortschritte d. Physik, xiii. (1857) p. 276.

<sup>†</sup> Donders, 'Bepaling van den Brandspunt-afstand van Lensen,' Versl. en Mededeel., xv. (1863) p. 402.

<sup>&</sup>lt;sup>‡</sup> Shellen, 'De Phakometer, ter repaling van foeus en centrum van henzen,' Maandblad voor Natuurwetenschappen, vii (1876) p. 23.

<sup>§</sup> Bessel, 'Astronomische Untersuchungen,' i. p. 137.

the focal length, and the (positive) lens is placed between them. Two such positions of the lens can be found for either of which the distance between the two conjugate foci is the same. Bessel gives a formula for deducing the focal length from the measurements of the various distances. The method assumes the distance between the Gauss points to be known beforehand, and therefore fails to give any information on the more difficult point to be determined. The advantages of the method are that no measurements have to be made from the curved faces of the lenses, and that none have to be made of the sizes of optical images.

(o) Oudemans'\* Method is simply the method of Bessel carried out by means of a special apparatus, consisting of a simple graduated bench, and hair micrometers. Oudcmans gives approximate formulæ for calculating the distance between the Gauss points, for insertion in Bessel's formulæ, but confesses that this procedure fails in the case of many lens-combinations.

(p) Hasselberg's<sup>†</sup> Method.—In applying Bessel's plan, Hasselberg employs as objects the real images of spectrum lines as formed in the focal plane of the eye-piece of a spectroscope. He compares to some hundredths of a millimetre, the performance of a Zciss's objective constructed of ordinary glass, but assumes the Gauss points by approximate calculation.

(q) MacGillavry's 1 Method.—This elegant method departs from Bessel's in that it requires measurements to be made of the respective sizes of object and image, as well as of the distance between them, and of the displacement of the lens between the two intermediate positions of adjustment to exact focus. But by this means all assumptions or estimates about the distance between the Gauss points are avoided. MacGillavry gives three formulæ from each of which this unknown quantity has disappeared by elimination; the true focal length being given in terms of the quantities directly measured, namely, from the relative sizes of object and image in the two positions, and the change in any one of the three measured lengths. Apart from the experimental difficulty of accurately measuring the magnification, Mac-Gillavry's method appears to be very satisfactory.

V. Method of Approximate Interior Unit Magnification.—(r) Cornu's § Method.—This is one of a group of possible methods in which the respective distances from their related principal foci of two conjugated points are measured, and the true focal length (which is their geometrical mean) is calculated from them; the peculiarity of Cornu's plan being that the two conjugate points employed are close to the two Gauss points, one of them being always interior to the lcns. The lens is temporarily marked with ink-lines upon its faces, and the experimental process consists in observing by a reading Microscope of sufficiently

\* Oudemans, 'Sur la détermination des distances focales des lentilles à court foyer,' Archives Néerlandaises, xiii. (1877) p. 149.
† B. Hasselberg, Bull. de l'Académie des Sciences de St. Pétersbourg, xxxii.

(1888) p. 142; and Beiblätter, xii. (1888) p. 782.

1 MacGillavry, 'De bepaling der focaal-afstanden van samengestelde optische stelsels.' Maandblad voor Natuurwetenschappen, v. (1875) p. 73. § Cornu, ' Détermination expérimentale des éléments principanx d'un système

optique,' Journal de Physique, 1re série, vi. (1887) p. 276.

long focus the positions in space of the principal focus of the lens, the marks on the nearer face, and the internal vertical image of the marks on the further face. From the distances thus measured, together with a measurement of the thickness of the lens, the true focal length, as well as the distance between the Gauss points, can then be calculated. In practice, the direct measurement of the thickness of the lens is avoided by the device of reversing the position of the lens and repeating the three readings from the reversed faces. M. Cornu describes an apparatus constructed for him by Duboseq for earrying out these measurements.

(s) Mebius'\* Method.—This is a modification of the method of Cornu for the particular case of negative lenses, and needs no extended notice here.

VI. Method of Obliquity of Rays. -(t) Moser's † Method. -This method is based on the principle that any ray which on entrance passes through the first Gauss point at any given obliquity with respect to the principal axis emerges with unchanged obliquity, but displaced, as if it had passed through the second Gauss point. To determine these points the experimental process consists in a series of approximations derived from measurement made of the magnification.

Of the various methods thus briefly reviewed, only those of Pendlebury, Meyerstein, MacGillavry, and Cornu fulfil the conditions of determining the values of both f and  $\kappa$  without double adjustments. Doubtless, each has its advantages for particular cases. Yet it appears worth while to follow out another method which seemed to possess some advantages over any yet suggested.

#### A NEW FOCOMETRIC METHOD.

In the new method of focometry which the author has devised, direct methods of measurement of lengths only are used; and double adjustments are avoided. The method consists in the direct determination, *firstly*, of the two principal foci by placing a transparent micrometric screen at each; and, *secondly*, when these have been found, the two symmetric points by moving the two screens by a double screw motion through equal distances until each is the image of the other. The true focal length (f) and the distance  $(\kappa)$  between the Gauss points are therefore given by simple subtraction of scale readings.

Choice of the Symmetric Points.—It is easy to show that in any determination of focal lengths, the most favourable position for an experimental measure of any two conjugate points is when these occupy the symmetric points; provided the experimental determination of the two conjugated foci is assumed to be of equal difficulty.

Let p and -q be the respective distances of the point-object and point-image from the two corresponding principal foei. Then by Newton's rule, we shall have

$$f^2 = p q; \tag{1}$$

where f is the true principal focal length.

<sup>\*</sup> Mebius, 'Détermination expérimentale des éléments principaux d'une lentille divergente,' Journal de Physique, 2me série, ix. p. 511.

<sup>†</sup> Moser, 'Methode die Brennweite und optischen Hauptpunkte von Linsen zu bestimmen,' Pogg. Annalen, lxiii. (1884) p. 39.

If in the determination of the lengths p and q we make errors of measurement, respectively  $\Delta p$  and  $\Delta q$ , there will result an error of  $\Delta f$  in the calculation of the focal length, having a value determined by the equation

$$(f + \Delta f)^2 = (p + \Delta p) (q + \Delta q), \tag{2}$$

or

$$f^{2} + 2f \Delta f + \Delta^{2} f = p q + p \Delta q + q \Delta p + \Delta p \Delta q.$$
(3)

Subtracting (1) from (3) and neglecting small quantities of the second order, we have :---

$$2f\Delta f = p\Delta q + q\Delta p. \tag{4}$$

Hence, divided by  $f^2 = pq$  we get

$$\frac{\Delta f}{f} = \frac{1}{2} \left( \frac{\Delta q}{q} + \frac{\Delta p}{p} \right), \tag{5}$$

or the percentage error in f is the mean of the percentage errors in pand q. Hence, since  $\Delta p$  and  $\Delta q$  are obviously of the same order of magnitude, if we write  $\Delta m$  for the arithmetical mean of them, and assume that each of them is equal to this value, we get from (4)

$$\Delta f = \frac{1}{2} \frac{p+q}{f} \Delta m \tag{6}$$

which shows that for a given mean error  $\Delta m$  and a given focal length, the error made in determining this focal length will be proportional to p+q. Hence those values of p and q which make p+q a minimum will make the error  $\Delta f$  a minimum. And, as  $pq = f^2$  is a constant for a given 'lens, it is obvious that the case of minimum value of p+q is when p=q; this being the case when the conjugate points are at the symmetric points.

The assumption made above that the experimental difficulty of determining the position of a conjugate focus is equal for conjugate foci in any position, is, however, hardly justified in practice, for in all laboratory experience it is admitted that it is more difficult to ascertain with precision the position of an image (real) which is remote from a lens than that of one near the lens. In fact, the experimental location of the image is mainly delimited by the sharpness of the crossing of the rays, and the tangent of the angle at which the extreme rays cross is inversely proportional to the distance from the lens. The aperturc of the lens then limits the accuracy of determination of foci at great distances. The larger the aperture the more accurately (assuming spherical aberration above) will be the delimitation of the foei; but the larger the aperture, the greater do spherical aberrations become. The error in determining q may arise at either end of the measurement; it is more likely to occur at the end most distant from the lens than at the principal focus. If it is assumed that the probable magnitude of an error  $\Delta q$ made in estimating the value of q is proportional to the distance of this focus from the lens, then we may write  $\Delta q$  as proportional to q + f, and similarly  $\Delta p$  as proportional to p + f. Substituting these values in (4) we get

 $2f\Delta f \propto p(q+f) + q(p+f)$ 

and dividing by f and collecting, we get

$$\Delta f \propto f + \frac{1}{2}(p+q).$$

This is still a minimum (for positive values of p and q) when p = q, or when the conjugate foci are taken at the symmetric points.

Principle of Focometer for determining the Focal Planes and Principal Planes of any given (Positive) Optical Combination, such as a Microscope Objective, or other Lens.—The abstract principle of this focometer was described as follows by the author two years ago, in a memorandum accompanying an application made to the Royal Society for a grant in aid of the construction of the apparatus :—

"Let A B (fig. 14) be the objective (or lens);  $H_1 H_2$  its principal points;  $F_1 F_2$  its principal foci; and  $S_1 S_2$  the two symmetric points





situated each at double the focal distance from the respective principal points,  $S_1 S_2$  are conjugate points, and the planes through them are planes of unit magnification.

"Suppose a parallel beam to be sent (from a lamp provided with a reticle in front of it and a collimating lens, all placed in air at somo convenient distance away) through A B from left to right. An image will be formed at  $F_1$ , thus dotermining that point. Then, with the same source removed to a distant point on the right, send a parallel beam from right to left, thus determining  $F_2$ . Small glass plates, having micro-meters cut on surfaces (respectively) nearest to  $H_1$   $H_2$ , and each provided with a reading lens behind, should be used to receive these images, and to ascertain their precise position in space. The said micrometers should be mounted on supports sliding along a suitable bench, over the middle of which the objective has been clamped in a special support. The micrometers, or, at least, one of them, should be so arranged that they can be thrown out of the axis laterally when not wanted. They should be provided with verniers to read off their positions on the bench. They should also be furnished with clamps, which, after each has been once set at its principal focus, will permit it to be clamped to a screw below on the bench. The distance from  $\mathbf{F}_1$  to  $\mathbf{F}_2$  is equal to 2f + x(where f is the true focal length and x the unknown distance between the two principal planes).

"Now, let there be a gearing, such as a right and left-handed screw, which will enable the observer to move the two micrometers from  $F_1$ and  $F_2$  at exactly equal rates outwards. When one of them arrives at  $S_1$  the other will at the same moment arrive at  $S_2$ , and this will be known by observing through the reading lens attached to one micrometer the inverted image of the other, coincident, but reversed in position (exactly as in Silbermann's old form of focometer). The equality of object and image in size—known by the fitting of the micrometer scales—will serve to check the correctness of the observation. The distance from  $S_1$  to  $S_2 = 4f + \kappa$ . Hence  $\kappa = 2 F_1 F_2 - S_1 S_2$ : and f = $F_1 S_1 = F_2 S_2$ . By measuring off backwards from  $F_1$  a distance equal to  $S_1 F_2$ , the point  $H_2$  is arrived at. Similarly  $H_1$  is arrived at, and these points can be marked off on the outside of the tube of the objective."

Description of the Instrument.—In accordance with the foregoing project, the author designed an instrument which he terms a Focometer. It was constructed by Messrs. Nalder Bros., of Clerkenwell, to whom the author is indebted for many useful suggestions embodied in the apparatus. The construction is shown in the accompanying figures.

The support for the lens or combination of lenses to be examined is fixed at the middle of a bench made of two parallel girders of gun-metal, each 670 mm. in length, placed vertically above one another, and secured together both at their ends and at the middle. The highest and lowest faces of this double girder are bevelled at  $45^{\circ}$ , and a scale of millimetres is divided along the front face at the upper edge. This girder frame is shown from the back in fig. 15, in end view in fig. 16. The support for the lens can be raised by a dovetail slide worked by a rack, or moved horizoutally transversely to the bcuch by another dovetail slide furnished with a screw motion, as shown in figs. 15 and 16.

The travelling supports for the micrometers are two solid pieces of brass, which fit over the bevelled edges of the girders and slide without any looseness of motion along the frame. Each bears a vernier to read off its position on the bench, and each is furnished at its upper point, as shown in fig. 15, with a horizoutal slide for fine adjustment, worked by means of a screw of fine pitch; the position of the horizontal slide being read off by means of a vernier against a short scale cut upon the face of the support. Except when the clamps described below are applied, each of these supports is so far free that it can be pushed along the bench by hand, but is fitted to slide so accurately that it cannot be shifted by any chance touch.

Between the parallel girders, running from end to end of the apparatus, is a double screw, the two halves being respectively right and left-handed, each accurately of a pitch of two millimetres. This screw, the function of which is to shift the two supports for the micrometers, is furnished at each end with a large milled head, and with a driving handle. The screw is of steel. It was constructed in two separate parts, which were then united by being securely riveted into a short cylinder of steel of larger diameter. This cylinder runs through a bearing in the central piece of the frame of the instrument, and is secured in position between two fixed collars of steel, which are seen edgeways in fig. 15. These collars are screwed up sufficiently tightly to prevent any end play. At the two outer ends the screw passes through two bearings in the end supports, which admit of longitudinal play so as to allow for any difference of expansion between the screw and the frame of the instrument.



The manner in which the driving motion is communicated from the screw to the two travelling supports is peculiar. A device was needed which would admit of the travelling supports being independently moved to any positions when not clamped to the screw, and of being clamped in any position to the screw, so as to be driven by it without backlash. These conditions were finally fulfilled in the following manner. Upon



each half of the screw is placed a massive gun-metal nut, about 50 mm. long, and of square section 25 mm. in the side. The lower face of this nut slides upon the upper face of the lower girder, and this prevents it from turning; it is also prevented from turning by the interposition, between its upper face and the lower face of the upper girder, of a long slotted rectangular flange of brass which constitutes part of a clamping bar. Each nut is bored out with a short cylindrical cavity. Within this are inserted a spiral spring of steel wire, and a second nut which, though capable of longitudinal play, is prevented from turning by the insertion of a key into a keywa⊽. The introduction of this second nut, with a strong spring between it and the main nut, is a mechanical device originally due

to Sir William Thomson, P.R.S., for preventing backlash. The slotted elamping bars mentioned above are of brass, each 176 mm. long. Each is, as may be seen from figs. 15 and 16, of L-shaped section, fitting over, and in front of the square nut. The upper horizontal flange which comes between the top of the nut and the under side of the higher girder is of accurate fit, and is slotted out to receive beneath it the head of a clamping pin. This pin, passing vertically through a projecting lug of the travelling support, enters the threaded shaft of a clamp-screw which bears at its top a milled head. When this is turned, the head of the clamping pin is raised and clamps the slotted bar to The vertically-situated flange of the lug of the travelling support. each of the L-shaped clamping bars fits accurately into the space between the upper and lower girders, and it also is slotted to admit tbrough it a clamping pin which projects horizontally from the square nut. A clamping screw with milled head screws on to this pin, and It is shown in fig. 16 projectclamps the slotted bar to the square nut. ing to the left. In order that the turning of the long screw may drive the two travelling supports, it is requisite that each should be clamped to its slotted bar, and that each slotted bar should be clamped to the square nut. The object of using such long clamping bars instead of mere short pieces is to enable the travelling supports to be elamped

when at unequal distances from the centro of the apparatus, the two square nuts being always situated symmetrically at equal distances from the centre.

The micrometers and other appliances for receiving the focal images are of three different kinds.

The first kind is shown on the left-hand support of fig. 15. It consists of a small bevel-edged disc of glass, fixed in the end of a narrow tube, and provided with a reading lens or positivo cyc-piece of about 38 mm. focal length. Upon the outer surface of the glass disc is ruled a scale divided into fifths of millimetres. This micrometric arrangement is convenient for receiving a focal image, as formed at the back of a microscopic objective, at a point at some distance down the tube in which the objective is mounted.

The second kind, shown in fig. 15, on the right-hand support, consists of a rather larger disc of glass, mounted in a metal rim, which is cut away at two places. Upon the front face of this disc, a micrometer scale in fifths of millimetres is also ruled, and one-half the disc is silvered over. This arrangement is convenient for use with larger lenses, and for service as an object, in which case it is illuminated from behind. When making determinations of microscopic objectives it is found convenient to use one micrometer of the second kind opposito the front of the objective, and one of the first kind at the back of tho objective; the picces being mounted as shown in fig. 15. Each of tho micrometers can be shifted laterally out of the lino of the lens under measurement, the arm which carries the micrometer being fastened to a collar furnished with a small handle, as shown in figs. 15 and 16. This collar is guided to move in a plane orthogonally to the optical axis of the apparatus by an adjustment of three abutting screws. These three abutting screws are not shown in fig. 16, but the heads of two of them are shown in profile in fig. 15; being situated behind the collar bearing, which receives the curved supporting stem of each of the two micrometers. These bearing-collars are provided with stops to enable the micrometers to be brought up accurately to thoir former position after having been thrown out laterally.

A third arrangement, not shown in fig. 15, is used only for largo lenses, and consists of a grooved metallic ring mounted on a curved arm upon the top of the travelling support. Over this grooved ring is stretched a piece of thin paper, which, after having a millimetre scale marked upon it, is rendered nearly transparent by the application of varnish. Two such micrometers are used—one at either end—when measurements are to be made upon camera lenses, and other lenses having a focal length of several centimetres.

The holder, fitted upon the central support, as shown in figs. 15 and 16, for carrying the lens, is cut with the Royal Microscopical Society's standard screw, so as to receive any microscopic objective. For other small lenses an adapter, provided with the same screw, is used, and the lens is temporarily fitted into the adapter by means of a cork ring. For large lenses a V-shaped appliance is substituted for the screw holder.

In order to know the position of the lens itself with respect to its focal and principal points, it is necessary to ascertain the scale-reading

corresponding to some fixed point on the lens, or on its attached mounting. This might be done by end-measurement, by bringing one of the micrometers up into actual contact with one pole of the lens; but such a mode of proceeding is inadvisable for several reasons. It was therefore decided, when the instrument was being designed, to adopt such a construction as would permit of direct determinations by means of a delicate plumb-line. With this object, the general form of double girder was adopted, so that the scale might be engraved on the front of the vertical face. By reference to the end elevation in fig. 16, it will be seen that the optical axis of the apparatus is arranged to be in the same vertical plane. Moreover, the micrometers are so arranged that in each case a plumb-line can be hung directly against the actual face of glass or paper upon which the micrometer is engraved. As plumb-line, a thin silk thread with a small leaden sphere at the end of it is used. When the instrument is properly levelled, the plumb-line can be applied to read off directly on the scale the actual position of any of the micrometers; and so by comparison with the readings of the verniers of the travelling supports is obtained the zero reading for use in future measurements.

It was consequently necessary to furnish the instrument with levelling screws. There, however, arose a small mechanical difficulty; for an instrument of this shape would not be very stable if provided with one foot at one end, and two near together at the other. It is therefore provided with four levelling screws, one at each end and two at the two ends of the central support. In practice, this arrangement is quite workable; and it is found convenient first to adjust the level of the girders lengthways by the end screws, then to adjust transversely by the other screws. The frame is so solidly made that there is no fear of racking it by unequal weight upon the four screws; the wooden top of a strong laboratory table is never so rigid as to make any fine adjustment necessary.

Mode of using the Focometer.-The mode of procedure in using the instrument is as follows :- The lens to be measured having been secured in the central support, it is then adjusted in position so as to be accurately in the axial line between the two micrometers. The clamps of the two travelling supports are left loose, being only applied when required. One of the micrometers (in practice, that shown on the left in fig. 15) is then thrown out laterally, and a beam of parallel light is thrown through the lens (from left to right, as seen in fig. 8), so as to form an image at the first principal focus. In practice, this is done as follows :- A compound lens, which is to be used as a collimator, is placed in direct sunlight, and at the focal point, where the image is formed, is placed a piece of ground glass, coarsely ruled with black lines. When this reticle is set in its exact position with respect to the collimating lens, the combination is placed at one end of a long room, about 40 feet long, with a paraffin lamp behind the reticle to illuminate it. The parallel beam thus issuing from the collimating lens is received on the focometer about 20 feet away.

The travelling support on the right (fig. 15) is then brought up by hand and adjusted so that the micrometer is approximately at the principal focus. It is then clamped to the screw, and, using a positive eyc-piece to aid the vision, the micrometer is accurately adjusted to the

126

focus either by turning the main serew, or by the fine-motion serew on the travelling support. This first adjustment having been made, the clamps are then unfastened, and the micrometer is thrown out laterally.

A parallel beam is then thrown in a similar fashion in the reverse direction through the lens, and the second micrometer is thrown into line and is brought by a similar process to the second principal focus.

The plumb-line is then applied to ascertain the position of some fixed point of the lens, and to read off the positions of the micrometers, which are replaced in their axial positions: or, if the zero readings of these are known, their positions are ascertained from the readings of their respective verniers.

These three readings having been made, both the travelling supports are clamped to their respective nuts on the main screw. The screw is turned so as to eause the micrometers to travel outwards. The observer, looking into the eye-pieee of the micrometer on the left, sees an inverted image of the other micrometer eome into view, and, as the screw is turned, the micrometers reach a certain position when both sets of dividing lines are in foeus in the same field without any parallax. This position ean be very accurately ascertained by shifting the eye slightly from side to side of the lens. The two micrometers now oecupy tho two symmetrie points, and their positions are observed either by plumb-line, or by the readings of their respective verniers.

The simple method of calculating by subtraction of scale readings the true focal length f and the distance between the two Gauss points, has been given above.

Results of Measurements made with the Focometer.—The following examples are given of measurements made on lenses. In all cases here recorded a red light was used, a ruby glass being interposed.

(1) Small Hemispherical Lens, 12 mm. aperture, by Cooke and Sons.—Front of hemispherical face taken as point of reference A. Preliminary experiments with plumb-line showed that  $F_2$  is 76.1 mm. behind zero of vernier  $Z_2$ , and  $F_1$  is 49.6 mm. in front of zero vernier  $Z_1$ .

Readings of verniers for principal foei are :--

$$\begin{array}{rcl} Z_1 &=& 356\cdot 55 & Z_2 &=& 199\cdot 20 \\ &-& 76\cdot 10 & &+& 49\cdot 60 \\ \hline F_1 &=& \overline{280\cdot 45} & F_2 &=& \overline{248\cdot 60} \\ \hline \text{Readings of verniers for symmetrie points are :--} \\ Z_1' &=& 371\cdot 52 & Z_2' &=& 184\cdot 17 \\ &-& 76\cdot 10 & &+& 49\cdot 60 \\ \hline S_1 &=& \overline{295\cdot 42} & S_2 &=& \overline{233\cdot 77} \\ f &=& \text{mean of } F_2 - S_2 \& S_1 - F_1 &=& \overline{15\cdot 03} + 14\cdot 97 \\ f &=& 15\cdot 0 \text{ mm.}; \text{ ff} &=& 66\cdot 6 \text{ dioptries.} \\ \kappa &=& 2 \left(F_1 - F_2\right) - \left(S_1 - S_2\right) \\ &=& 2\cdot 05 \text{ mm.} \\ H &=& 12\cdot 63\cdot 6 \cdot H \text{ at } 265\cdot 65 \text{ of scale} \\ \end{array}$$

 $H_2$  is at 263.6;  $H_1$  at 265.65 of scale. A is plumbed at 262.65. Hence first principal point is 0.95 mm. within the front of the hemispherical surface. (2) Substage Condenser (single lens), by Beck.—Flat upper face of lens taken as point of reference A.

$$\begin{array}{rll} A &= 251 \cdot 8 \\ Z_1 &= 356 \cdot 32 \ ; & Z_2 &= 196 \cdot 5 \\ &-76 \cdot 1 & + 49 \cdot 6 \end{array} \\ \hline F_1 &= 280 \cdot 22 & F_2 &= 246 \cdot 1 \\ Z'_1 &= 370 \cdot 54 & Z'_2 &= 182 \cdot 17 \\ f &= \text{mean of } Z'_1 - Z_1 \text{ and } Z_2 - Z'_2 &= 14 \cdot 28 \text{ mm.} \\ \mathfrak{f} &= 65 \cdot 4 \text{ dioptries.} \\ H_2 &= F_1 - f &= 265 \cdot 94 \\ H_2 &= F_2 + f &= 260 \cdot 38 \\ \kappa &= H_1 - H_1 &= 5 \cdot 56 \text{ mm.} \end{array}$$

(3) Coddington Lens, reputed 1 in. focal length. — Summit of ono curved face taken as point of reference.

 $\begin{array}{l} \mathbf{A} &= 265 \cdot 8 \\ \mathbf{B} &= 280 \cdot 9 \\ \mathbf{F}_1 &= 299 \cdot 3; \ \mathbf{F}_2 &= 247 \cdot 2 \\ \mathbf{S}_1 &= 325 \cdot 6; \ \mathbf{S}_2 &= 220 \cdot 8 \\ f &= (\text{mean of } \mathbf{S}_1 - \mathbf{F}_1 \text{ and } \mathbf{F}_2 - \mathbf{S}_2) = 26 \cdot 35 \\ \text{if } &= 37 \cdot 95 \text{ dioptrics.} \\ \mathbf{H}_1 &= \mathbf{F}_1 - f = 272 \cdot 95 \\ \mathbf{H}_2 &= \mathbf{F}_2 + f = 273 \cdot 55 \\ \kappa &= \mathbf{H}_1 - \mathbf{H}_2 = -0 \cdot 60 \text{ mm.} \end{array}$ 

The Gauss points are crossed, and close together.

(4) Objective 1-in., by R. and J. Beck. A (front of lens) at 248  $\cdot$  2. F<sub>1</sub> = 279  $\cdot$  88 F<sub>2</sub> = 241  $\cdot$  98 S<sub>1</sub> = 300  $\cdot$  09 S<sub>2</sub> = 221  $\cdot$  75  $f = 20 \cdot 22$  mm.  $f = 49 \cdot 46$  dioptrics. H<sub>1</sub> = 259  $\cdot$  67 H<sub>2</sub> = 262  $\cdot$  21  $\kappa = H_1 - H_2 = -2 \cdot 54$  (Gauss points crossed).

(5) Objective 1/4 in., by R. and J. Beck.—Back focus too deep in tube to use ordinary micrometer; used a  $1\frac{1}{2}$ -in. objective instead, as auxiliary lens, to explore focus.

A (front of lens) at  $252 \cdot 4$   $F_1 = 274 \cdot 88$   $F_2 = 252 \cdot 14$   $S_1 = 281 \cdot 20$   $S_2 = 246 \cdot 2$   $f = 6 \cdot 13 \text{ mm.}$   $\mathfrak{f} = 163 \cdot 1 \text{ dioptries.}$   $H_1 = 268 \cdot 75$   $H_2 = 258 \cdot 27$  $\kappa = H_1 - H_2 = 10 \cdot 48 \text{ mm.}$ 

128

(6) Objective reputed 16 mm., by Zeiss ("apochromatic").--(Used auxiliary lens as in preceding for back focus.)



 $A = 233 \cdot 9^{7}$   $F_{1} = 417 \cdot 8$   $F_{2} = 151 \cdot 0$   $S_{1} = 547 \cdot 7$   $S_{2} = 20 \cdot 45$   $f = 130 \cdot 22 \text{ mm.}$   $f = 7 \cdot 68 \text{ dioptries.}$   $H_{1} = 287 \cdot 58$   $H_{2} = 281 \cdot 22$   $\kappa = 6 \cdot 36 \text{ mm.}$ 

(9) Aplanatic Condenser for Lantern, constructed by R. and J. Beek on Herschel's formula.—A taken at front of mount on coneave side.

 $\begin{array}{l} A = 237 \cdot 5 \\ F_1 = 365 \cdot 5 \\ F_2 = 193 \cdot 3 \\ S_1 = 435 \cdot 7 \\ S_2 = 121 \cdot 8 \\ f = 71 \ 0 \ \text{mm.} \\ \text{ff} = 14 \cdot 8 \ \text{dioptries.} \\ H_1 = 294 \cdot 2 \\ H_2 = 264 \cdot 3 \\ \kappa = 29 \cdot 9 \ \text{mm.} \end{array}$ 

1892.

(10) Camera Lens (small landscape), maker unknown.—A taken at rim of mount at front end.

$$\begin{array}{l} A = 247 \cdot 2 \\ F_1 = 416 \cdot 2 \\ F_2 = 136 \cdot 7 \\ S_1 = 534 \cdot 2 \\ S_2 = -1 \cdot 3 \\ f = 138 \text{ mm.} \\ \text{ff} = 7 \cdot 248 \text{ dioptries.} \\ H_1 = 278 \cdot 2 \\ H_2 = 274 \cdot 7 \\ \kappa = 3 \cdot 5 \text{ mm.} \end{array}$$

No complete examination of any lens for rays of different colours has yet been made, but it has been found that lens No. 4, examined above for red light, gives with green light a different value for  $\kappa$ , though the lens is sensibly achromatic at the principal focus.

The most interesting results obtained so far are the facts that in so many compound lenses the Gauss points are crossed, the first point being beyond the second. And, in the ease of onc lens (No. 7), a Reichert's objective, the distance between these two points is found to exceed the distance between the two principal foci. It seems to be a necessity with all wide-angled compound lenses that the aberrations can only be reduced to a minimum by widely separating the constituent lenses, with the result that the optical centres of the combined lens are considerably displaced past one another.

Since the bulk of the foregoing investigations were carried out, the authorities of the Kew Observatory have decided upon undertaking the testing of camera lenses, and issuing certificates of merit. In this work they have had the benefit of the advice of Captain Abney, F.R.S., than whom no one is better able to advise as to what is desired for photographic purposes. This is an excellent beginning, but it is curious that in neither of the certificates issued is any information given as to the position of the optical centres, or their distance apart. The "differences in focal length" for red and violet rays are given, but whether this means difference in true focal length, or difference in position of focal plane, is not stated. What a photographer wants is not agreement in focal length, but in focal plane, which is a very different matter, Also in the "A" certificate issued from Kew, it is proposed to state the optical distortion at 25° from the axis, "including astigmat'sm." But whether this means that the test is to include one for cylindrieity so as to give the direction of the axis of astigmatism in the focal plane, or whether this term is being misused to denote spherical aberration, does not appear. Certainly no really astigmatic lens could be tolerated for an instant in photography, as it would result in all vertical lines being out of focus when all horizontal lines were in focus, or some similar defect.

I notice in a recent most admirable article on photographic lenses, in 'Nature,' by Mr. A. Mallock, the term astigmatism is also used for the distance between the primary and secondary foci as produced by oblique rays. But this is not astigmatism at all, and has nothing to do with the

130

eylindricity of the lens, the defect which produces astigmatism. What Mr. Mallock applies this term to is spherical aberration pure and simple. I pointed out in 1889, in my article in the 'Philosophical Magazine,'

how the focal power of a lens is the product of a number depending only on the properties of the material into the sum of the two curvatures of its faces, or, in the case of thick lenses of a more complex quantity depending on the thickness of the lens as well as its eurvatures. And in order to facilitate calculations I devised a special form of spherometer —the dioptrie spherometer—(fig. 19) which measures the curvatures of the surfaces directly in dioptries. This was also constructed for me by Messrs. Nalder Bros. To apply this to any simple lens one merely takes





the two readings, adds them, and multiplies by a constant \* which depends only on the material. For erown glass the constant is 0.54; and for flint glass it varies from 0.64 to 0.78 according to density.

\* This is equal  $\mu - 1$ , where  $\mu$  is the refractive index; or  $(1 - h) \div h$ , where h is the velocity-constant (relatively to air).

Since I published this, a still more handy device has been brought out by the Geneva Optical Company, of whom Messrs. Botwright and Grey, of Clerkenwell, are agents in this country. It is known as a 'Lensmeasure,' and is depicted in fig. 20. It is the invention of Mr. Brayton, and consists of a simple form of spherometer with a multiplying-hand to read off the curvature on a dial. But the latter, instead of reading off the mere curvature of the surface, has the readings of its dimensions already multiplied by the proper constant for crownglass, namely, 0.54; so that the dioptries of a lens are found simply by adding the reading of the two sides.

I will conclude by expressing my satisfaction that the British Association has seen its way to appoint a committee on the subject of the measurement of lenses. With Prof. Carey-Foster as chairman, and with Captain Abney, of South Kensington, and Mr. Whipple, of Kew, as members, such a committee onght to be able to effect some real progress in the spread of scientific methods, and so help forward the industry.

The paper was succeeded by a discussion.

Mr. G. M. WHIPPLE said he had been much gratified at hearing the reference which had been made to the work done at Kew in regard to lenses, for it ought to be better known that an attempt had been made in this country to create an optical laboratory. He was also very glad to know that the Technical Institute at Finsbury was doing similar work, and he felt sure that with the appliances they had there and the leisure at their disposal-not of Prof. Thompson, but of the senior students -many most interesting questions in optics might be dealt with, and he hoped solved, which could not be accomplished by those who had other duties allotted to them. The measurement of lenses for Microscopes had not come within the scope of their operations at Kew; their work was the examination of telescopes for the royal navy, binoculars, gun directors, a class of telescope not much known to the general public which had been recently brought out, besides smaller glasses used in the ordinary work of navigation. Again, the sextants which were employed by cadets must bear a certificate, and this had led to increased accuracy in make. There were also certain minor instruments examined, of which he need only mention one, which required a plane surface. The grinding of a plane glass surface was a somewhat difficult operation, and it had not been much required until recently, when they had been introduced and their use rendered compulsory for cadets as artificial horizons in working their sextants. This was a matter of economy to save the waste of mercury, which was somewhat considerable, when mercurial artificial horizons were employed. Plane surfaces of blackened glass These things had been going on for were therefore introduced instead. some time, but of late the Kew Committee had undertaken the examination of photographic lenses, and he must add to the names of the gentlemen who had been mentioned as laying down the lines on which the work should proceed, that of Major Darwin, formerly director of the photographic department of the School of Military Engineering. He had now retired from the Royal Engineers, and had thrown himself thoroughly into this question of testing photographic lenses. He seemed to have consulted all the authorities he could get at, and had made some very ingenious and beautiful pieces of apparatus for the purpose, amongst
them being a modification of Grubb's method of determining the focal length of lenses. It was much larger than Prof. Thompson's apparatus, being adapted to test lenses of 6 iu. diameter and 30 in. focal length, and was about 5 ft. long and the same height. It proved very satisfactory in use. He regretted that the word astigmatism had remained on the prospectus and was kept in use, for it undoubtedly was wrong, but still it was a very convenient term to employ, and he did not know what could be substituted for it, and it was apparently understood by users of photographic lenses. There were other terms as regarded distortion and dispersion, which were not altogether correct, and should give place to a more accurate terminology. The point with regard to focal planes, which formed such an important feature in microscopic work, had not come before them in connection with photographic lenses, nor did they use it at all in testing telescopes aud binoculars.

Mr. T. R. DALLMEYER said it was very gratifying to find that at last the efforts which were being made to obtain perfection in optical work were to be subjected to critical and scientific examination, which must result in really good work being appreciated. He was particularly interested in what he understood to be a new theory in treating light, as explained in the early part of the paper, and he wished Prof. Thompson had developed it further. One of the main things which opticians had to do was, after calculation, to do what he might describe as grinding a lens on paper, and this work, by ordinary processes, was very laborious. He imagined that if the process of which Prof. Thompson had only given the brilliant idea, were carried out with regard to the central pencils, that process of going through the mill in lens-grinding would be greatly facilitated. From the one or two hints which had been given, the method seemed to be simplicity itself. There were one or two other methods of obtaining the nodal points besides those which had been mentioned. He should like to ask if, in particular constructions-one of which he was deeply interested in-one of the nodal points was only radially outside the lens, would such an application be suited to the measurement of the lens as regarded its focus. Hc understood that, for taking long measurements, such an instrument would be hardly applicable, but that it was chiefly confined to the measurement of lenses where the nodal points were either contained in the instrument, or were very close to it.

Mr. CONRAD BECK said he also had been extremely interested in the paper, because he remembered his fearful struggles in endeavouring to work out lenses on the English system, and the delight with which he hailed the Gauss and the German system of reckoning both the signs and the principal planes. Those who had endeavoured practically to work out lenses on the ordinary system, as given in "Parkinson," and such books, would agree with him as to the enormous difficulties there were, which were entirely got rid of when the proper geometrical method of reckoning the signs, and the complete theory of the Gauss points were brought into work. As Mr. Whipple had said, the chief importance of the nodal points was with reference to microscopie work, and in that case there were some very awkward and difficult cousiderations. In old days, when the achromatic Microscope was first introduced, it was understood that some sort of reasonable magnifying scale should be adopted, and an arbitrary scale was taken, in which the inch meant that it magnified a certain amount with a certain tube-length. But nobody knew where the tube-length was measured from. An American gentleman the other day published a paper in which he tabulated all the various tube-lengths as measured by various English and Continental manufacturers, and they varied most cnormously. But, even supposing a definite tube-length to be taken, the difficulty still remained, because in order to get a magnifying power which should be in any way consistent with changed eye-pieces and object-glasses, it was necessary that low-power object-glasses should be mounted in enormously long tubes and high powers in very short tubes. When using a low power you then had to have it a long way from the object to begin with, and that difficulty was increased by mounting it in a tube 3 in. or 4 in. long, which in the case of a 5 in. would be simply preposterous. Then, again, supposing that, for the sake of scientific accuracy, such a plan were adopted,-what could be done in the use of a binoeular Microscope? With the binocular Microscope it was essential to have the object-glass as near as possible to the binocular prism, whereas if lenses were mounted on the principle suggested, the low powers, which are the very powers used for binocular work, ought to be mounted a long way away from the tube of the Microscope. Until, however, this plan was adopted, no really true method of magnification could possibly be established. As a matter of fact opticians at present were making their low-power lenses very much higher in power than they ought to be in order to obtain this standard magnifying power which was adopted as an arbitrary seale. For instance, a modern 4-in. objective was nothing like 4 in.; it was nearer 3 in., because its nodal point was too far up the Microscope. It was put up to a much higher power to produce the same magnifying power in connection with the same eye-piece. This plan got over the difficulty tolerably, but when you changed the eye-piece you began to find that although with one particular eyc-picce the magnifying seale was tolerably constant-and could be made absolutely constantwhen you ehanged the eye-piece it did not affect different powers in the proper ratio, and the seale was thrown out. Mr. Whipple said the nodal points were not of so much importance in photographic and other optical lenses, and he was quite right in saying that their position was not of nearly so much importance, but it was extremely important some means should be adopted which eliminated the distances between the nodal points in measuring the focal length of optical instruments.

Prof. THOMPSON, in reply, said Zeiss's way of getting over the difficulty about tube-length was not quite as Mr. Beck had stated, because Zeiss distinctly attempted to regulate the depth to which the eye-picees were to be plunged down, so that they should not overlap in the same way as in the old arbitrary scale. Whether he was successful in carrying that out with very low powers he could not say. In reply to Mr. Dallmeyer's question, he said that the present instrument would not measure lenses of more than 6 in. focal length. Mr. Whipple seemed to give no hope that the practical user of lenses would be content to change the misleading term astignatism; but what were you to do if you earne across a lens which had two defects, and both were called astigmatism? If they meant different things you must give a different name to one of them, and he thought you ought to give a different name to the thing which was not astigmatism. Mr. Whipple and the Kew authorities ought to invent a name,\* and then every one would be obliged to adopt it.

The CHAIRMAN, in proposing a vote of thanks to Prof. Thompson, said that this paper constituted a really important contribution to the knowledge of optical measurements. The instrument which had been shown and explained promised to be very useful indeed in the aetual examination and specification of lenses. Hitherto there had been no accurate method which was easily applicable for finding the constant on which the action of any lens, and, still more, a combination of lenses, depended. In the ordinary treatment of the properties of lenses in this country, even in scientific text-books, one got no further than dealing with lenses which were not infinitely thin, but which were dealt with as though they were, and he hoped the paper would have a great effect in widening the ordinary optical discussions, and bringing the ordinary theory more nearly into accord with actual practical experience. As yet they had hardly got beyond Newton's optics. What Gauss introduced long ago, the idea of the virtual thickness of the lens, had scarcely been recognized in all its importance in this country, though it was of the utmost value in facilitating the statement of the properties of lenses. One small point in the paper which he thought of some importance, was the introduction of a term for what he believed had no name before, which was often referred to in foreign writings as the vertex, and which was here called the pole. Prof. Thompson had already made important contributions to optical theory; and he would refer especially to some papers of his which appeared two years ago in the 'Philosophical Magazine,' where he showed how very simply the properties of lenses could be expressed by the method he had hinted at in the beginning of this paper -by speaking of the eurvature they impressed on the wave front of a beam of light passing through them. In those papers he had treated all the ordinary cases from that point of view with extreme simplicity and beauty, and he hoped this method would be adopted generally in the ordinary treatment of optics.

The Dioptrical Principles of the Microscope.<sup>†</sup>—Prof. G. Macloskic considers that the formulæ for calculating the path of light through a centered system of lenses is unnecessarily complex. He has tried a simple formula in Matthiessen's 'Dioptrik,' and finds that it ean be extended to all cases, so that a single formula ean be need to determine the focal lengths of lenses, doublets, objectives, eye-pieces, and of the entire optical system of a Microscope or telescope. For the refraction of a ray through a surface from a medium with index  $n_0$  to another with index  $n_1$ , we have the two focal lengths

$$f_1 = \frac{\frac{n_0}{n_1}r}{\frac{n_0}{n_1} - 1} \qquad \qquad g_1 = \frac{\frac{n_1}{n_0}r}{\frac{n_1}{n_0} - 1}.$$

\* The author has since suggested the term *aplanatism* as a suitable name to denote this quantity. † The Microscope, xi. (1891) pp. 209–15.

The formulæ giving the focal length of a lens are

$$f = \frac{f_1 f_2}{f_2 - g_1 + t_l}$$
 and  $g = -f = \frac{-g_1 g_2}{f_2 - g_1 + t_l};$ 

where  $f_1 g_1$  are the refractions for the first surface and  $f_2 g_2$  those for the second, and  $t_l$  is the thickness of the lens. These focal lengths are measured from the principal planes. The author designates the three segments into which the lens is divided by these planes by the names *anteplane*, *interplane*, *postplane*, and the interval between the second principal plane of one lens and the first principal plane of the following lens by the term *transit*.

The formulæ for determining the principal planes are

$$a_1 \text{ (anteplane)} = \frac{-f_1 t}{f_2 - g_1 + t}$$
$$a_2 \text{ (postplane)} = \frac{-g_2 t}{f_2 - g_1 + t}.$$

The above formulæ give the focal lengths, &c., of a single lens, but by altering slightly the signification of the letters they also serve for the combination of two lenses to form a doublet. In this case  $f_1 f_2$ , &c., denote the focal lengths of the individual lenses instead of those of the surface refractions, and t denotes the transit distance instead of the thickness of the lens. The same formulæ then serve for combining the doublets into an objective, and finally for finding the dimensions and focal lengths of an imaginary lens equivalent to the whole Microscope.

To illustrate the application of the formulæ the author takes the case of a Microscope composed of an objective consisting of three doublets and the ordinary Huyghenian cyc-piece. Each of the doublets C, B, A consists of a plano-convex flint lens  $(n = 1 \cdot 6)$  combined with a double convex crown lens  $(n = 1 \cdot 5)$ . The thickness of the three flint lenses is 1/2, 2/3, and 3/4 mm., and that of the three crown lenses 1, 4/3, and 3/2 mm. respectively, with radii of curvature 1, 4, and 10. The front vertex of the second and third doublets coincides in each case with the second principal plane of the underlying doublet. The eye-piece consists of two convex-plane crown lenses, with convex side downwards. The lower one is 3 mm. thick, and has radius of curvature 40, while the upper is 2 mm. thick, with radius of curvature 30.

Taking as an example the case of the crown lens of the front doublet C, we have

$$f_{1} = \frac{-r}{n-1} = \frac{-1}{1\cdot 5 - 1} = -2. \qquad g_{1} = \frac{nr}{n-1} = 3.$$

$$f_{2} = -\frac{nr}{n-1} = -3. \qquad g_{2} = \frac{r}{n-1} = 2.$$

$$\therefore f_{c} = \frac{f_{1}f_{2}}{f_{2} - g_{1} + t} = -\frac{6}{5}.$$
Anteplane = 2/5 and postplane = -2/5.

136

Similarly, for the flint lens we have  $f_f = 5/3$  with Anteplane = 5/16 and postplane = 0.

In the figure the principal planes of the flint lens are represented by  $f_1 f_2$ , those of the crown lens by  $c_1 c_2$ , and those for the whole doublet by  $d_1 d_2$ .

The transit distance for the two lenses  $f_2 - c_1 = 2/5$ .

Hence the first principal focal length of the doublet is

$$f = \frac{f_f f_c}{f_c - g_f + t} = \frac{30}{13}.$$

The same processes are applied to the other doublets A and B, which are then combined into a low power objective, whose principal planes have the positions marked A  $B_1$  and A  $B_2$ . This is then combined



with the front doublet to form the total objective, whose principal planes are marked  $O_1 O_2$  in the figure. Similarly, the equivalent lens for the eye-piece is determined, and finally the cye-piece is combined with the objective. The transit distance in the last case depends on the tubelength, and was 220.8432 for the Microscope used by the author. Tho total focal length was found to be .623, while the lower principal plane was 1.18 below the front vertex  $V_0$  at the point  $M_1$  in the figure, and the upper one at 6.794 above the eye-piece. The anterior focal length is positive, and its focus is represented by F.

The Effect of Curvature of the Cover-glass upon Micrometry.\*--Dr. M. D. Ewell writes :---" Inasmuch as by far the greater number \* Proc. Amer. Soc. Micr., xii. (1891) pp. 79-83. of observations with the Microscope are made upon objects under a coverglass, it becomes an important subject of inquiry whether microscopic measurements are sensibly affected by the curvature of the covers. Especially is this true as to such experts as profess to be able to identify blood by the measurement of the red corpuscles. I have not been able to find in the literature any investigation of this subject, and having in the Cronin case been called as an expert with reference to the identification of blood by micrometric measurement of the red corpuscles, I made the observations herein recorded, not with a view to publication, but in order to be able to come to a correct conclusion as to one factor in that case.

In the comparison of long standards this question has no importance whatever, inasmuch as they are always uncovered; but inasmuch as most objectives in common use are corrected for covered objects, or are usually so used, the value of one division of the micrometer used with such objectives on covered objects must be determined from a micrometer having its lines covered either permanently or temporarily.

It is obvious that sensible curvature of a cover-glass interposed between the object and front of the objective must affect the amplification of the objective. If such curvature is spherical this effect will be symmetrical; if the curvature is irregular it must distort the object, so that the interval between two objects will appear to be different according to the orientation of the cover-glass.

Several methods are available for testing the curvature. It may be tested by viewing with a telescope the image of some regular object, such as a building reflected from the cover-glass. It may also be tested by accurately focusing a telescope upon some object, such as an artificial star, and then interposing the cover-glass to be tested between the object viewed and the objective of the telescope. If the cover is flat and if its sides are parallel, such interposition will have no effect upon the image other than very slightly to diminish the amount of light received from it. If the cover-glass is spherical it will change the focus of the telescope, which must be refocused in order to obtain a clear image of the object observed. If the cover-glass has irregular curves the image will also be distorted. If the cover-glass is prismatic the image will be displaced laterally.

A very much more sensitive method than either of the above is that by the observation of the Newton's rings, caused by placing the coverglass upon a flat surface. For this purpose I obtained one of Mr. Brashear's justly celebrated flat test surfaces; but having found upon trial that the second method above described was sufficiently sensitive for my purpose, it was used exclusively in the observations hereinafter described. Tested by this method, I may state that nearly all the cover-glasses in common use possess either regular or irregular curva-Tested by the method of Newton's rings, I am informed by ture. Mr. Brashear that it is practically impossible to find one perfectly flat. I have myself examined and tested a large number furnished me by the courtesy of the Bausch & Lomb Optical Company and the Palmer Slide Company, as well as some purchased of Mr. Zeiss of Jena, and have found a very few that by the second method above described were sensibly flat. By far the greater number possessed quite sensible curvature.

138

My method of determining whether the curvature of the cover-glass sensibly affected micrometric measurements was carefully to measure the same space under identical conditions, except as to the cover-glass, and to compare the results. For this purpose a high-angled dry objective was employed, as being more likely to be affected by a variation in the cover-glass than a homogeneous immersion or low-angled objective. The cover-correction was made as perfect as possible and not changed thereafter.

The measurements were all made with a Bausch & Lomb first-class 1/6 objective (cover-correction = 0 + 3 + 1/2 + 2/10), a Bulloch filar micrometer, a Smith's vertical illuminator, manufactured by Mr. W. H. Bulloch, and a Bulloch stand, 'Professional No. 2.'

The space measured was the second 1/100 mm. of 'Centimeter A,' counting from the first line of the 1/100 mm. band. The temporary covers, except as otherwise noted, were 16/100 mm. in thickness, and all except numbers 1, 7, and 9 were taken at random from covers purchased in the ordinary course of trade or furnished by the courtesy of the Bausch & Lomb Optical Company and the Palmer Slide Company. No. 1 was so nearly flat that its curvature, if any, could not be detected by the second telescopic test above described. Nos. 7 and 9 were covers ground and polished to order by Messrs. Bausch & Lomb, with the express purpose of making them as nearly flat as possible, but with the effect of making them more curved than covers usually are which have not been thus treated, experience showing that the covers are so easily warped that they cannot by ordinary methods be ground flat.

The measurements are recorded in terms of divisions of the micrometer of which 1 division =  $0.0732 \mu$ . The number at the head of each series refers to the temporary cover used in the series.

October 6, 1889.	
No. 1 (flat cover).	No. 4.
131.4 div.	131·7 div.
131.2 "	3.9 "
133.0 "	1.0 "
132.0 "	2.9 ,,
133.0 "	1.0 "
Mean: 132.1 "	Mean: 132.1 "
No. 2.	No. 5.
132.4 div.	131.9 div.
132.5 "	1.0 "
131.6 "	1.8 "
130.5 "	1.2 "
131.9 "	1.7 "
Mean: 131.8 "	Mean: 131.5 "
No. 3.	No. 6.
130.0 div.	131.7 div.
3:3 "	1.7 "
$1 \cdot 9$ ,	2.5 "
0.7 ,,	2.4 ,,
1.9 "	1.1 "
Mean: 131.5 "	Mean: 131.9 "



	Mean.	Difference from Mean.
1. Flat cover	132·1 div.	- 0·1 div.
2	131.8 "	+0.2 "
3	131.5 "	+0.5 ,,
4	132.1	-0.1
5	131.5	+0.5
6	131.9	+0.1
7 B & L	132.5	- 0.5
8 Zeiss	131.2	+0.8
9  B & L	132.3	- 0.3
10 No 1 second time	139.7	- 0.7
10. No. 1, second time	199.7	- 0 7 ,,
11. Cover 8/100 mm	132.7 "	- 0.7 ,,
12. No. 1, third time	131.6 "	+0.4 "
Meau of all 12 series	132.0 ,,	

Mean of all, excepting the series with the thin cover (No. 11),  $= 131 \cdot 9$  div.

Mean of the eight series excluding the three series of measurements with No. 1 and the series with the thin cover  $= 131 \cdot 9$  div.

Mean of the three series of measurements with No. 1, the flat cover  $= 131 \cdot 1$ .

An inspection of the above measurements discloses the fact that the differences from the mean in the case of the three series in which the flat cover was used are as great as when other covers were used, and

140

that the mean of all the measurements using the flat eover is sensibly the same as with the other covers (some of which possessed a eurvature quite sensible), the difference being only  $0.02 \mu$ , which is too small to measure.

It was intended to make a much larger series of measurements, but the pressure of other work prevented.

The foregoing are, however, sufficient to warrant the conclusion (to which there may possibly, in the case of very bad covers, be some exceptions) that using good eover-glass, such as is furnished by reputable dealers in this country, the most, if not all, of which is manufactured by Chance, the influence of the curvature of such covers is practically *nil*.

This is not the conclusion to which the writer had come from *a* priori reasoning; but it is the only one warranted by such facts as have been observed. Further observations will be presented in a future paper."

Simple Method of Finding the Refractive Index of various Mounting Media.—Mr. E. M. Nelson communicates the following:—Provide two precisely similar equi-convex lenses, whose identical refractive index  $\mu$  and radii r are known, and cement them together with tho mounting medium whose refractive index has to be determined.

Now measure F the principal focus of the combination, then the refractive index of the mounting medium.

$$\mu' = 2 \ \mu - 1 - \frac{r}{2 \ \mathrm{F}}.$$

It is convenient to make the radii of the equi-convex lenses 2 in. Then

$$\mu' = 2 \; \mu \; -1 \; - rac{1}{\mathrm{F}} \; .$$

Some examples might be of interest.

Let the refractive index  $\mu$  of the two equi-convex lenses be 3/2, and suppose that the combination has no focus, that is, that it behaves like a piece of plane glass, then

$$\mathbf{F} = \ \infty, \ \ \frac{1}{\mathbf{F}} = 0,$$

and

$$\mu' = 2 \ \mu - 1 = 2 \cdot 0.$$

If the principal focus of the combination F = +2 then

$$\mu' = 2 \ \mu - 1\frac{1}{2} = \frac{3}{2},$$

or the same as the equi-convex lenses.

But if the principal focus of the combination F is negative, it must be measured in the same way as a concave spectacle lens, viz. by neutralizing it by a positive lens of equal focus. If F is negative the sign before the fraction will be changed.

Example, let

Then

$$F = -2$$

$$\mu' = 2\mu - 1 - \frac{1}{-2} = 2\mu - 1 + \frac{1}{2} = 2 \cdot 5.$$

The above method gives a greater range of readings for indices varying from 2 to  $2 \cdot 5$ , and consequently more accurate results than the simpler one of filling up a plano-coneave lens with the medium and covering it with a piece of plane glass. The formula for this latter plan being

$$\mu' = \mu + \frac{r}{\mathbf{F}} \cdot$$

The radius of the concave r might with advantage be made 2 in., then

$$\mu' = \mu + \frac{2}{\overline{F}} \cdot$$

If  $\mu = \frac{3}{2}$ , and  $F = \infty$ ,  $\mu' = \frac{3}{2}$ ; if F = 4,  $\mu' = 2$ ; and if F = 2,  $\mu' = 2 \cdot 5$ .

#### (6) Miscellaneous.

Experiments on the Diffracting Structure of Striated Musclefibre.\*—Dr. O. Zoth has made a series of experiments on the diffraction of striated muscle-fibre similar to those first undertaken by Ranvier.

He supplements the simple method of observation employed by Ranvier with the method of Abbe, by which the diffraction spectra are observed in the Microscope, and uses the following arrangement. The source of light was at first a vertical slit, 10 mm. long and 1 mm. broad, in the screen of an argand burner, but later this was replaced by a zircon thread ignited by a Bunsen flame. Behind the Bunsen burner is a black background, and in front of it a black diaphragm with rectangular aperture, which serves to permit of the measurement of the bright line and to cut off extraneous light. The light from the source was reflected from a plane polished steel mirror, and a real image of the bright line was formed above the Abbe condenser. With the ordinary distance of 30-40 cm. between line and mirror the condenser (Zeiss 1.2 Ap.) throws an image of the line above the object. The Microscope, with low magnification (Zeiss A, eye-piece 2), is first adjusted on the object; the body-tube is then gradually raised until the image of the line is clearly defined, when on both sides of this direct image the diffraction spectra become simultaneously visible.

For the measurement of the distances of the individual spectra it was considered sufficient for the author's purpose to determine by means of an eye-piece micrometer the linear distances between the centre of the undiffracted image and the centre of the yellow in each spectrum.

The sartorius muscle of the frog was made the chief subject of experiment, and the numbers obtained by the above method in this case were compared with those resulting from similar observations on insect muscle. The arrangement employed was: Zeiss objective A, eye-piece III., with micrometer 5 mm. in 50 divisions, body-tube 155 cm., condenser 1.2 Ap., distance of source of light from the centre of the mirror 35 cm. The numbers obtained in divisions of the micro-

<sup>\*</sup> S.B. Akad. Wiss. Wien, xcix. (1890) pp. 421-43.

meter were: for the frog's muscle 27-32, for the muscle of Dyticus 20-24, for that of Hydrophilus 13.5-16.5.

The muscle in these experiments was prepared by Ranvier's method, which consisted in scraping it down on both sides with the scalpel. The thinnest of the lamellæ thus prepared might contain several superposed layers of fibre, so that it became of importance if possible to observe the diffraction effect of the cross striation of the individual fibres.

For the purpose of such experiments, teased preparations were made of muscle which had been kept for a long time in 93 per cent. alcohol, and these were mounted in a mixture of equal parts of glycerin and The method of observation was the same as before, except that water. the Abbe condenser was lowered so far that the image of the source of light was projected beneath the plane of the object. Owing to the different thicknesses of the object-holders for the various preparations, it was necessary in each case to readjust so that the image of the source produced by the condenser should always be at the same distance beneath the plane of the object. This was effected by first adjusting the Microscope upon the object, then lowering the body-tube always by the same amount (102 divisions of the micrometer screw = 0.48 mm.), and finally raising or lowering the condenser until the image of the source became sharply defined. In the following table are given the results of measurements on different muscle-fibres.

Group.	No.	Object.	Measure- ments.	Limits.	Mean.
I. Grating on glass.	$\begin{array}{c}1\\2\\3\end{array}$	1 mm. in 100 divisions ", 500 ", ", 1000 ",		-	$\begin{array}{c}2\\10\\20\end{array}$
II. Old alcohol-muscle; teased preparations in equal parts of glycerin and water,		Hylobius abietis Hydrophilus piceus Scarabæus laticollis Vespa Crabro Melolontha vulyaris Dyticus marginalis Rana escul, stretched Felis domestica Rana esculenta	4 4 4 4 4 4 4 4 4 4 4 4 4	$\begin{array}{c} 2\\ 2 \cdot 5 - 3 \cdot 5\\ 3\\ 3 \cdot 5 - 5\\ 4 \cdot 5 - 6\\ 6 - 8\\ 6 - 8\\ 10 - 12\\ 11 \cdot 2 - 12\end{array}$	$   \begin{array}{r}     2 \\     2 \cdot 9 \\     3 \\     4 \cdot 2 \\     5 \\     7 \\     7 \cdot 1 \\     11 \cdot 1 \\     11 \cdot 9   \end{array} $

By comparing the numbers obtained for the muscle-fibres with those for the gratings, we have as mean distance of the striæ, since these are inversely proportional to the linear distances of the spectra, for the unstretched frog's muscle  $\mu 2 \cdot \frac{10}{11 \cdot 9} = 1 \cdot 68 \mu$ , for the stretched muscle  $\mu 2 \cdot \frac{10}{7 \cdot 1} = 2 \cdot 82 \mu$ , for the muscle of *Hydrophilus* 7  $\mu$ , for that of *Scarabæus*  $6\frac{2}{3}$   $\mu$ , and for that of *Hylobius* 10  $\mu$ . These numbers, however, are based on the assumption that the muscle-fibres are similar to simple gratings with equal parallel and equidistant spaces. Such an assumption is approximately correct for the frog's muscle in which tho striæ Z (Rollett's notation) are only visible with great difficulty or not at all; but it cannot be made for many other striated muscle-fibres, in which besides the striæ Q there are also recognizable, separated from Q by isotropic layers, the more strongly refractive finer striæ Z, and even another set N. The following table contains the results in microns for the three insect muscles, of the direct measurement of the distance of the striæ by the use of the eye-piece micrometer (Zeiss imm. 1/18, 1 division =  $1.08 \mu$ ).

		H.	ha.	hi.	tropic striæ.
Hydrophilus	••	 7.5	3.8	$3 \cdot 5$	Q, Z.
Scarabæus		 7	$3 \cdot 25$	$3 \cdot 75$	Q, Z.
Hylobius	**	 $13 \cdot 5$	$6 \cdot 5$	7	Q, N, Z.

The approximate agreement of these results with those obtained by the diffraction phenomena would seem at first to warrant the idea that one of the cross striations, either Z (and N) or Q, is without influence on the total diffraction effect. Such a supposition, however, is not justifiable, and a great mistake would be made if it were attempted to draw conclusions from it in the direction of the Abbe diffraction theory; for the diffraction phenomena resulting from the complicated structure of insect muscle could hardly be expected to follow the laws of diffraction of a simple grating.

In a grating prepared for the author by Dr. Steeg of Homburg, the distances of the dark bands could be varied. This grating consists of



two superposed glass plates, each of which carries a fine division in 0.1 mm. The upper plate can be moved over the other by means of a micrometer screw, and can also be rotated so as to bring the lines in the two plates parallel. Fig. 22 shows the diffraction phenomena

corresponding to thirteen different arrangements of this grating. The black oblongs represent bright spectra, the hatched ones those with weakened intensity.

For the purpose of investigating to what extent the peculiar character of an abnormally diffracting grating has influence on the observed diffraction phenomena, eight photographic gratings on silver bromide gelatin plates were prepared. These gratings had the following dimensions in millimetres :--

Grating.			1	Vidth of clear space.	V	Vidth of opaque band
I.	••	••		0.07	••	0.158, 0.035
II.				0.175	••	0.158
III.			••	0.298		0.035
IV.	••	••		0.084		0.084
v.	••			0.158, 0.035	••	0.07
VI.				$0 \cdot 14$	••	0.193
VII.			••	0.114		0.023
VIII.				0.07, 0.035		0.28, 0.035

Their arrangement may be better understood from fig. 23, where they are represented under a magnification of twenty.

Grating I. is an attempt to represent the cross striation of a *Hydrophilus* magnified about 48 times. Nos. II. and III. are produced from No. I. by removal in the one case of the narrow, and in the other



of the broad dark bands. No. IV. is a simple grating, with spaces and bands the mean of those of No. I. No. V. is a negative of No. I. In No. VI. the width of the dark bands corresponds to the sum of the width of a broad and narrow band, and the width of a space to double the width of a space of grating I. In No. VII. the distance between the equally wide spaces is equal to the mean distance between the spaces in I. Lastly, grating VIII. is an attempt to represent a *Hylobius* muscle with strike Q, N, and Z.

The diffraction phenomena produced by these gratings were observed in a dark room. The distance between the spectra was measured by means of a scale 10 cm. long suspended just above the aperture of the diaphragm in front of the zircon thread. This scale was illuminated from the side by an argand burner, and had white and black divisions alternately at distances of 1/4 cm. The gratings were placed at a distance of 150 cm. from the source of light, and the intervals between 1892. the spectra observed by looking through them at the bright line were measured on the scale.

Grating I. gave 5 spectra on both sides at intervals of one scale division.

II. gave 8 spectra on both sides at intervals of one scale division.

III. 10 spectra visible, interval 1 scale division.

IV. 3 spectra, 2 scale divisions.

V. 7 spectra, 4/5 scale division.

VI. 4 spectra, 1 scale division.

VII. 4 spectra, 2 scale divisions.

VIII. 7 spectra, 3/5 scale division.

Grating on glass in 0.1 mm., 6 spectra, 3.5 scale divisions.

Thus, so far as the interval between the spectra is concerned, gratings II. and III. behave in precisely the same way as I. A difference between them, however, was noticed in the case of a fainter photographic proof in which the dark bands were not quite black: for grating I. the spectra 2 and 4 were seen to be considerably reduced in intensity, whereas for gratings II.–IV. no such effect was observed.

A muscle of Hydrophilus observed under the Microscope by use of a higher objective (Zeiss D) showed a similar reduction in the intensity of spectra 2 and 4.

The result of these experiments is to show that no definite conclusion as to their grating arrangement can be drawn from the diffraction phenomena obtained from the complicated striated muscle-fibre of insects. No consequences, therefore, as regards their resolution in the direction of the Abbe diffraction theory can be drawn from the observation of their diffraction effects.

The author has repeated Ranvier's experiments on the diffraction phenomena of living frog's muscle in different conditions of expansion and contraction, by using the Abbe method of observation.

Fig. 24 represents the object-holder which served for the preparation of the fresh sartorius or hyoglossus of *Rana esculenta*. In the

F1G. 24.



middle of a plate of vulcanite O is a rectangular opening, above which is cemented a glass plate G with rounded edges. On each side of this are two binding-screws for the wires of an induction coil Through a hole in each of these binding screws passes a steel rod which carries at one end a small hook H and at the other a spring clip of platinum P. These rods are held firm by the screw S', but can be taken out and reversed if necessary. Over a small pulley at one end of the holder a thread attached to a scale-pan can be passed. This thread, after removal of the right steel rod, is fastened to the hook on the left and runs exactly above the plane

146

of the glass plate through the boring of the right binding-serew. Lastly a cover-glass D mounted in a metal frame is brought over the glass plate. The frame has on each side projecting pieces which fit into vertical grooves so that the cover-glass, either by its own weight of 1.45 grm. or by means of the two serews S, can be pressed down upon the preparation on the glass plate.

For observations on the unstretched muscle, the muscle is simply laid upon the glass plate, and covered by the cover-glass. For investigating the effect of stretching on the diffraction phenomena, the muscle is fastened to the hooks H, or preferably one end is fastened to the steel hook on the left, while the other is attached to the string which passes over the pulley to the scale-pan, in which different weights can be placed so as to vary the tension. For the observation of the diffraction phenomena during contraction and tetanus, the muscle is stretched in the same way. To conduct the eurrent a fine wiro connects the binding-screw through which the thread passes with the hook by which the thread is attached to the muscle. In these experiments the general arrangement for observing the spectra was the same as before.

The distances of the first spectrum from the undiffracted image in the case of the sartorius were as follows: unstretched, 35 divisions; weighted with 2 grm., 30 divisions; weighted with 10 grm., 25 divisions; for a hyoglossus at its maximum tension, 17 divisions.

Compression of the muscle has no marked influence on the distance of the spectra. Contraction of the muscle causes the spectra to approach one another. No displacement of the spectra occurs on exciting the muscle when at its maximum tension.

The late Mr. G. F. Dowdeswell, F.R.M.S.—The late Mr. Dowdeswell, who was some time a member of our Council, and for several years a constant attendant at our meetings, died in October last at the age of 56 years. His career was somewhat varied, for after an education at Eton and Magdalene College, Cambridge, he entered the army; ho served through the Mutiny and the Chinese campaign of 1860-6. The later years of his life were devoted to Histology and Bacteriology, and he published on both subjects a number of important papers, partly in our Journal, in the Proceedings of the Royal Society, and elsewhere. Among the subjects in which he specially interested himself we may note the structure of spermatozoa and the "cholera bacillus," Mr. Dowdeswell was also a Fellow of the Linnean and Chemical Societies.

Good Advice!—The Editors—Professors E. D. Cope and J. S. Kingsley—of the 'American Naturalist' are often asked what journals of biology a college with limited funds should take. As may be supposed, they place their own journal first; "next in importance is the Journal of the Royal Microseopical Society." It is not for us to deny this.

\* Amer. Natural., xxv. (1891) p. 895.

#### β. Technique.\*

# (1) Collecting Objects, including Culture Processes.

New Method of Studying the Development of Micro-organisms and the Mutability of their Characters and Properties. $\dagger - Dr. S.$ Délépine writes as follows :--- "Those who have followed the discussions which have taken place between the partisans of the constancy (Koch, Zopf) and those of the mutability of the pathogenic bacteria (Davaine, Naegeli, Pasteur, Buchner), know what stress has been placed on the impurity of cultivations, the pathogenic properties of which seemed to have altered. It is evident that one of the simplest ways to solve this vexed question would be, instead of studying the mixed products of the germination of a number of spores, to isolate one spore, and follow its development through all its stages, and the development of successive generations of organisms all derived from the same original spore and cultivated in various media. If it were possible to follow thus the history of one spore and its progeny, it would only be necessary, in order to obtain definite results, first to consider the complete series of morphological changes which occur, when the descendants of the same individual are cultivated severally in various media, then to connect certain physical and chemical alterations of the various media with stages of development, modified and unmodified, and finally to find how the properties of the organism at each developmental stage, are, or are not, modified by external circumstances. I had already attempted to carry out this plan by means of a dilution method, such as that used by Brefeld and others, since early in 1881, when working at the organisms of suppurating mucous membranes, a work which I gave up owing to the failure of the methods I was using then, and the special difficulties connected with the subjects. I was, however, already then able to satisfy myself that phenomena analogous to those of karyokinesis were of constant occurrence in multiplying bacteria and gave rise to many appearances which have been observed by others, though not explained fully yet.

About the middle of last year, whilst studying the development of certain pathogenic moulds and other parasites, I felt again the need of following closely the development of single organisms. I failed by plate and drop cultivations to obtain the results I wanted, partly owing to the effects of liquefaction of certain media, or of the mobility of others, partly also owing to the form assumed by drops. I was then led to adopt a new mode of cultivation which, although not perfect in many of its details, has yielded results which so far have been satisfactory, and some of which have been exhibited this year at a meeting of the Pathological Society (May 5th). The principle of the method is to inclose a thin layer of the nutrient media between two parallel plates, so as to force the organism to grow in definite directions. Owing to the effects of capillarity the most flucnt nutrient media become, so to speak, fixed, provided evaporation be prevented, and they become as available as the

<sup>\*</sup> This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes; (4) Staining and Injecting: (5) Mounting, including slides, preservative fluids, &c.; (6) Miscellaneous.

<sup>†</sup> Internat. Journ. Micr., iii. (1891) pp. 339-44. See Lancet, June 13th, 1891.

The method can of course be varied in many ways; but solidified ones. one of the simplest, and one of those which I have used with satisfactory results, is the following. At each end of a glass slide  $(1\frac{1}{2}$  in. by 3 in.) a narrow slip of glass is fixed A A. This, as will be seen later on, is to act as a support. (The surface of the slide on which these slips are fixed will be called the upper one hercafter.) Three small drops of scaling-wax are dropped on the upper surface of the heated slide (any other thick cement, solid, and emitting no antiseptic vapours, at the temperature of the body, may be used instead of scaling-wax.) These drops will be used to support a cover-glass C,  $1\frac{1}{4}$  in. in diameter, at a certain distance above the slide, and thereupon must form the apices of a triangle capable of being inscribed in the circumference of such a cover-glass. Before placing the drop of fluid on it, the slide must be thoroughly sterilized in the flame of a Bunsen burner, or otherwise. (The sealing-wax does not interfere with this process.) Then the slide is inverted or placed under a thoroughly sterilized plate. cover-glass C, 11/4 in. in diameter, is sterilized also, and the surface which is to be next to the slide is carefully protected from the access of any germ or dust. On this surface a very small drop D of sterilized material may be placed, and this drop touched with a wire charged with a few organisms. A number of cover-glasses being prepared in this way, they may be examined over a sterilized plate with a pretty high power, inoculated surface downwards, and not in contact with the supporting slide, which must also be thoroughly sterilized, until a drop is found to contain the number of organisms wanted. Instead of a drop, a streak E E can be used, according to the nature of the organisms investigated. On the upper surface of the sterilized slide a drop of sterilized nutrient or other medium is deposited by means of a perfectly sterilized pipette. The size of the drop depends on the thickness one desires to give to the preparation or the surface one intends The diameter of the inclosed film should, in order to prevent to cover. contamination, never be more than 3/4 in. when the cover is not more than  $1\frac{1}{4}$  in. in diameter. (I often use larger covers and slides, but this in most cases has no advantage.) The centre of the side of such a drop may now be inoculated (in case the cover has not reviously inoculated). Then the cover is placed over the It should be well supported by the three drops of sealing-wax, been previously inoculated). drop. and should not at this stage flatten much the drop underneath. A heated rod is then applied successively over the three drops of sealing-wax, until the inoculated fluid has spread evenly over a certain surface under central parts of the cover; the preparation is then ready for the incuba-It has, however, to be kept in mind that, owing to the free access tors. of air to the surface of the inoculated fluid, it is necessary to keep the preparation in a moist chamber. The extremely small size of these inoculated slides allows of a large number being packed in an extremely small space. Before incubating the preparation it is necessary to select out of the micro-organisms which have been sown into the fluid one or several, the position and relation of which are carefully noted.

For this purpose, divided cover-glasses or slides, or, as I prefer, a finder, can be used. I have in this way followed the development of bacteria and spores of the pathogenic Pyrenomycetes for days and weeks.

.

I have devised many improvements for regulating the thickness of the inclosed film, and making its thickness perfectly even, but these are not necessary to the success of the method, and their description would obscure its main object. Now it will appear to many that this is nothing else than a drop cultivation, and to others that it is a film or plate cultivation. It is all this, but I claim that it is something more; for by using an interlamellar film, as I feel inclined to call it, the free surface of the medium is limited to the space existing between the two glass lamellae used, whilst in ordinary plate, film, or drop cultivations, the surface in contact with the air is very extensive. By the interlamellar method, a side view, so to speak, of the cultivation is obtained; by the drop method, a surface view. By the interlamellar method organisms placed at various distances from the free surface of the



Fig. 25.—Slide with interlamellar film, ready for incubation. A A, Slide rests. B B, Drops of sealing-wax supporting the cover. C C, Cover-glass compressing a drop of nutrient material. D, Very small drop of gelatin or other material containing a few spores.

Fig. 26.-Cover-glass, with very small central drop inoculated.

Fig. 27.-Cover-glass, with a small central streak EE containing microorganisms.

medium can be followed in their development step by step,\* a thing utterly impossible in drop cultivation. By the interlamellar method it is possible to follow certain chemical changes occurring along a growing filament or colony extending in a direction which can always be determined; † this is impossible to the same extent in drop cultivations.

By the interlamellar method it is possible to follow for weeks the development of the same individual, or group of individuals, even in the midst of a fluid material; this cannot be done for any considcrable length of time in drop cultivations; I feel therefore justified in claiming for the method some advantages. I wish, however, to

\* In this way the branching of many bacilli can be demonstrated. This branching, which is supposed by most authorities not to exist among the Schizomycetes except in a spurious form, can by this method be easily demonstrated. I had, it is true, been led to believe in its existence from the study of organisms grown differently, but by this method branches may be seen to arise from definite filaments. Dr. Slater, who has kindly made many observations with me for the study of this point, has obtained results confirming entirely my views.

<sup>+</sup> By using media containing substances capable of forming insoluble compounds with the products of the metabolism of micro-organisms, the gradual formation of these metabolic products can be followed and demonstrated. Thus I have lately been able to demonstrate the formation of oxalic acid out of various substances, such as gelatin, starch, gum arabic, and possibly cellulose. I have been able to show that the formation of this acid begins only when growing filaments have free access to air—a fact of great significance in connection with Pasteur's teachings. state clearly that it has disadvantages of a serious kind when the objects in view are not thoso which I have tried to explain, and therefore I do not offer this new method as anything more than a help to those who may try to solve some of the questions to which I have referred.



Diagrammatic representation of eleven interlamellar cultivations in a moist chamber, showing the disposition which I have adopted both for this method and other forms of film or plate cultivations. A, Outer basin, containing a thin layer of water (W) at the bottom. B, Covering basin, with a flat bottom, allowing a series of moist chambers to be piled one above the other in the incubator. C, Inner bell preventing condensed water falling upon the slide. D, Plates supported by pieces of cork. E's, Slides.

On some future occasion I hope to be able to give further details regarding the modifications which have been suggested to me by circumstances and the general nature of the results I have obtained."

Cultivation of Bacilli of Asiatic Cholera.\*—Dr. Hueppe cultivated cholera bacilli in egg albumen, and by this method so virulent did the bacilli become that an injection of the culture into a guinea-pig produced toxic results in a few hours, and in a few days toxines were developed in sufficient quantities to kill the animal, while formerly forty days frequently elapsed before the death of the creature, even if it died at all. The active agent was globulin, which developed only in albumen and in no other substance. This toxin caused nephritis in most cases. The author believes the toxin of cholera to be a pepton. The aerobic bacilli of cholera were very resistant to hydrochloric acid, and thus adapted to pass through the stomach into the intestine.

Glass Cover-tube as Substitute for Cotton-wool Plug.<sup>†</sup>—As substitute for cotton-wool, and thereby avoiding many of the inconveniences of this system of plugging test-tubes, Dr. Schill advocates a simple device which he has adopted for two and a half years with satisfactory results. In principle it consists in covering one test-tube with another. The cover-tube is about two-fifths the length of the cultivation-tube, and both are made quite straight, and not lipped, so that they are easily slipped the one over the other, the interspace being about as thick as a

\* International Congress of Hygiene and Demography, 1891. See Lancet, ii. (1891) p. 376.

† Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) pp. 657-9 (1 fig.).

sheet of paper. The author employs them in two sizes-one 16 cm. long by 15 mm. in diameter, the other 18 cm. by 25 mm.; the former for stroke and puncture cultivations, and for small quantities of fluid media; the larger size for potato cultivations, for large quantities of solid or fluid media, and for purifying agar.

For facility of sterilizing in dry or moist heat, for cultivations in any kind of media and with any kind of gaseous environment, the author states that this simple device gives very encouraging results, and obviates at the same time many of the inconveniences of cotton-wool.

Apparatus for filtering Gelatin.\*-Dr. Schill points out that gelatin may be filtered much more rapidly than by the ordinary filter by means of a vessel the bottom of which is perforated by a number of holes-so many more filters, in fact. The bottom of the vessel is of course covered with filter-paper.

The simplest way to make the apparatus is to knock off the top of a preserve-tin or jam-pot, and make holes in the bottom with an awl or gimlet. The holes are made inwards. The bottom is then covered with filter-paper, the edge of which is made to lap up along the side of the vessel. The filter-paper is supported by a double layer of book-muslin, from which all grease has been carefully removed, and this is fixed round the filter by means of a rubber band.

For hastening the process of filtration, atmospheric pressure may be made use of. In this case the filter-vessel must have a top with a hole fitted with a caoutchouc stopper perforated for the passage of a glass tube. To the outside end of the tube is applied a funnel, while the lower end reaches very nearly to the bottom of the filter. Consequently as soon as a thin layer of gelatin forms at the bottom of the filter, the air above is compressed, and this accelerates the flow of the fluid. In other respects the apparatus is the same as the previous one. The filter apparatus may be made of wood or tin, but best, of course, of glass or porcelain.

Graduated Capillary Pipette for measuring very small quantities of fluid.<sup>†</sup>—Dr. G. Gabritschewsky has devised a pipette by which it is possible to remove a deposit from 0.001-0.1 ccm. or any intermediate quantity of fluid. The instrument is made on the same principle as that used in the enumeration of red corpuscles, and fitted at the end with a rubber tube clipped by a screw-clamp. The pipette, the exact form of which is given in an accompanying illustration, is chiefly intended for estimating the number of bacteria in a fluid. The instrument is to be cleaned after each time of using with dry heat, alcohol, ether, and water.

## (2) Preparing Objects.

Study of Development of Cephalopods.<sup>‡</sup>-Mr. S. Watase separated the blastoderm from ova, which he had artificially fertilized and kept alive, by killing the egg at any given stage of division by a mixture of sea-water, acetic and osmic acids, as recommended by the Hertwigs for their macerating fluid. The osmic acid may be reduced in quantity or

- \* Centralbl, f. Bakteriol, u. Parasitenk., x. (1891) pp. 659-60 (2 figs.).
- <sup>†</sup> Tom. cit., pp. 248-9 (1 fig.).
  <sup>‡</sup> Journal of Morphology, iv. (1891) pp. 249 & 50.

dispensed with. It was found most satisfactory to remove the ovum from the action of the reagent as soon as the translucent protoplasmic germ-disc turns whitish opaque, which takes place very quickly, and to complete the separation of the blastoderm in dilute glycerin. The separated blastoderm may afterwards be stained with dilute Schneider's acetic carmine, or with Ehrlich's hæmatoxylin, and then mounted in glycerin; or it may be left unstained, for most things can be satisfactorily made out without any staining, and by avoiding this unnecessary process of *technique* the risk of injuring or dislocating the blastoderm is greatly lessened. Treatment of living eggs with Perenyi's fluid for a few seconds is excellent for the surface study of cleavage; it turns the protoplasmic portion of the ovum opaque yellow, and brings out the cleavage furrows distinctly, while the rest of the egg remains translucent as before.

Mode of Investigating Bryozoa.\*—Mr. C. B. Davenport found that the Gymnolæmata present many difficulties to finer technique; their chitinous covering is often very thick, and there is frequently, in addition, a calcarcous skeleton. When the latter is present, piero-nitric acid mixed with sea-water is a fairly good fixing reagent; when it is absent, hot eorrosive sublimate is the most serviceable. Extreme caution must be taken in transferring the objects through the grades of alcohol, so as to avoid the collapse of the ectocyst; the author made use of the chloroform-paraffin method of imbedding in order to make the transfers more gradual. The best staining agents were alcoholic dyes like Kleinenberg's hæmatoxylin and Mayer's cochineal, though Ehrlich's hæmatoxylin was often used with success.

Method for demonstrating Structure of Spinal Cord and Cerebellum.<sup>†</sup>—Prof. A. Van Gehuchten, from a series of observations made on the spinal cord and cerebellum, confirms the original statements of Ramón y Cajal, who in his researches followed the technique devised by Golgi with some slight modifications.

The spinal cord and cerebellum, in the embryonic, newly born, and adult conditions, of several kinds of mammals and also of the fowl, were used for the purposes of these observations.

The author's practice was to place small pieces of nervous tissue in a mixture of 3 per cent. bichromate of potash and of 1 per cent. osmic acid in the proportion of 4 parts bichromate to 1 part osmic acid. The pieces, several millimetres thick, usually remained in the mixture for two to two and a half days at the ordinary temperature, but fixation can be hastened by keeping them at a temperature of  $35^{\circ}$  to  $40^{\circ}$  for thirty to forty hours. On removal the pieces are rapidly washed in distilled water, and then immersed in the 3/4 per cent. nitrate of silver solution, whereby a precipitate of chromate of silver is deposited in the nervous tissue. The addition of one drop of formic acid to 100 cem. of the silver nitrate solution considerably aids reduction, the precipitation commencing immediately on immersion of the pieces in the bath, wherein they remain for at least twenty-four hours, but a still longer stay is not harmful, provided light be carefully excluded.

\* Bull. Mus. Comp. Zool., xxii. (1891) p. 3.

† La Cellule, vii. (1891) pp. 81-122 (4 pls., 55 figs.).

On removal from the silver solution the pieces are placed in 96 per cent. spirit for fifteen to twenty minutes, and then for a quarter of an hour in absolute alcohol. The next step is to soak them in a dilute solution of celloidin for a quarter of an hour, then fix them to a piece of cork, after which they are hardened in 70 per cent. spirit, so that the sections may be cut about an hour after being removed from the silver solution. The sections are next immersed successively in alcohol and creosote, cleared up in oil of turpentine, and mounted in xylol-dammar. The dammar should be dried as quickly as possible by keeping the preparations in an incubator at 40° for twenty-four hours.

Method for demonstrating Structure of the Cerebral Cortex.\*-In his researches on the cerebral cortex, Prof. Ramón y Cajal used small mammals in the embryonic and recently-born conditions. The procedure adopted was the rapid method of Golgi. The author found that the most favourable period for obtaining a good colouring of the nervous elements was not the same for all the animals employed; for example, in mice the optimum varied from the 8th to the 25th or 30th day, while in the rabbit the favourable period was found to be from the 1st to the 15th day after birth. The time required for hardening in the osmic-bichromate mixture was two, three, to five days, in recentlyborn rabbits, guinea-pigs, and cats, but this is only a rough estimate, for the time required varies for each animal and for every stage of This caution holds good not only for the fixation but development. also for the colouring of the different constituents of nervous matter, some elements requiring a longer time than others.

In order to insure a certain result it was found necessary to always carry on the procedures at a constant temperature. In winter the author worked at a temperature of  $25^{\circ}$  to  $26^{\circ}$ , this being maintained by a stove with a thermo-regulator.

The size of the pieces to be hardened should not exceed half a centimetre to a volume of 25 to 30 cubic centimetres of the mixture.

Occasionally no reaction takes place or is very imperfect, owing to the proper period for impregnating having been exceeded. In this case a successful result may be sometimes obtained by reimmersing the preparations for another 24 or 36 hours in the silver solution.

When colouring the superficial elements of the brain, it is very important to prevent any deposit of chromate of silver crystals on the surface. This may be avoided by the devices suggested by Martinotti and by Schrwald, and also by leaving the pia mater and arachnoid on the cortex, or by covering the cortex with a thin layer of the fresh blood of the animal.

The addition of one or two drops of a concentrated solution of chromic acid to the solution appears to aid the colouring of the collateral fibres. It is certainly advantageous in spinal cord, more especially if the vertebral column has to be cut together with the cord, since it helps to dissolve out the inorganic matter from the bone.

The author used the original mixtures for the chrom-osmic acid and silver solutions, not modifying them in any way, nor did he adopt

\* La Cellule, vii. (1891) pp. 125-32 (3 pls., 19 figs.).

the suggestions of Greppin and Obregia in preserving the impregnated sections, for he has found that the chromate of silver deposit and the chloride of gold, while fixing the protoplasmic expansions and the large axis-cylinders, act very unfavourably on the delicate collateral branchlets, causing them sometimes to disappear altogether.

Permanent Preparations of Aleurone and its inclosed Substances.\* —Dr. F. Krasser recommends the following methods for the differentiation of the ground-substance, crystalloids, and globoids in aleurono grains. A very favourable object is *Ricinus communis*.

1. Picrin-eosin.—Sections are fixed with picric acid dissolved in absolute alcohol, and the excess removed by absolute or very concentrated alcohol, and are then stained by cosin dissolved in absolute alcohol, cleared by oil of cloves, and set up in Canada balsam dissolved in chloroform. The staining is accomplished in a very few minutes. The preparation shows the ground-substance dark-red, the crystalloid yellow with a sharp outline, the globoid nearly colourless or reddish.

2. Picrin-nigrosin.—The section is placed in a saturated solution of picric acid in absolute alcohol, in which nigrosin has been dissolved to saturation; the staining is continued until the ground-substance becomes blue. The preparation is then washed with absolute alcohol and placed for a very short time in oil of cloves, and set up in Canada balsam. The ground-substance is blue, the globoid colourless, the crystalloid yellow-green with a sharp outline.

Very good preparations of the crystalloids alone can be obtained by staining with eosin after first dissolving off the ground-substance and globoids by a very dilute aqueous solution of sodium phosphate.

"Microplyne" and "Microzete."<sup>†</sup>—M. L. G. Chauveaud describes two appliances which he finds of great use in the preparation and examination of sections of vegetable tissues. The "microplyne" is a small glass funnel with a delicate perforated disc of platinum placed across the tube. On this disc is first of all placed a layer of powdered glass; then the sections of tissue which have been properly prepared, then another layer of powdered glass; and the staining reagent is then allowed to filter through the powdered glass on to the section; the excess filtering away through the lower layer of glass. The watchglasses containing the stained sections are then placed in the "microzete," a table illuminated from below by movable double black and white mirrors, and with a movable lens above by which to examine them.

# (4) Staining and Injecting.

Some Methods of treating Nerve-tissues.<sup>‡</sup>—Dr. W. C. Krauss writes :—" The aims to be sought after in the study of microscopy are not alone those looking to the perfection of the instrument nor those which bear upon the selection of the specimen. The preparation of this specimen, and more especially the method of staining, is as important as the specimen itself. American microscopists are more intent upon the former; the European microscopists pay more attention to the latter.

\* S.B. K. K. Zool.-Bot. Gesell. Wien, May 29, 1891. See Bot. Centralbl., xlviii.
(1891) p. 282. † Ann. Sei. Nat. (Bot.), xiv. (1891) pp. 16-24 (3 figs.).
‡ Proc Amer. Soc. Micr., xii. (1891) pp. 116-9.

Careful study and experimentation with the different dyes have led to the compounding of stains which have enabled the Microscope to reveal many of the mysteries of histology and pathology. The introduction of the anilin dyes and their successful employment by Koch, led to the discovery of the bacillus of tuberculosis, although the existence of this organism had been prophesied by writers years before its discovery. Golgi's silver nitrate method has advanced our knowledge of the ganglion cells of the cortex of the brain, and perhaps at some day the mystery of their poles may be revealed through some simple method of staining. The importance, therefore, of the methods of staining is not to be overlooked or undervalued in the microscopical examination of tissues, and their successful employment may lead to the discovery of new facts and data of inestimable value in advancing the present status of our science.

Among some of the recent methods employed in neuro-histology and neuro-pathology, perhaps none are so important and satisfactory as the Weigert method and the Pal modification of this method. Both methods are restricted to the examination of nerve-tissues, more especially of the central nervous system, where the gradation between white and grey matter is distinct and prominent.

Both methods require hardening in Müller's fluid, or simply in a saturated solution of potassium bichromate. A recent writer in 'Neurologisches Centralblatt,' Dr. Minor, of Moscow, finds that if sections of the brain and cord are subjected to the action of the positive pole in the bichromate solution, hardening will take place in three, four, or five days. After dehydrating and decolorizing in alcohol for some days, the preparations are ready for the imbedding mass. I have always preferred celloidin for imbedding nerve-tissues, and find that it is an excellent agent. It is prepared by allowing several sheets of celloidin to dissolve in equal parts of sulphuric ether and 99 per cent. alcohol. The preparations to be imbedded are placed in 99 per cent. alcohol for 24 hours, transferred to equal parts of 99 per cent. alcohol and sulphuric ether for another 24 hours, then placed for 24 hours in the celloidin solution, fastened upon corks, and are then ready for cutting.

The sections being more or less delicate and very friable, it is necessary to protect them during their passage through the various stages of the process. For this purpose collodion, photoxylin, or dextrin may be used, the *modus operandi* being as follows:—Allow some of the mass to flow over a glass slide, so that a thin film remains; dry; then transfer the sections on to this prepared slide, and pour some of the mass over the sections, allowing all superfluous quantities to drip off. After a few moments the slide may be moistened in alcohol, when the sections imbedded in collodion can be removed and handled with impunity.

The Weigert method, first described by Prof. Weigert, of Frankfort on Main, in 'Fortschritte d. Medicin,' 1884, p. 190, and 1885, p. 236; also 'Zeitschrift f. Wissenschaftliche Microscopie,' 1885, p. 490, and 1886, p. 480, requires the sections to be placed in an aqueous-saturated solution of cuprum acetate diluted with an equal amount of water for 24 hours, in a brood oven, or 48 hours in the open air. They are then washed in 60 per cent. alcohol a few hours and placed in the Weigert staining fluid :—0.75-1.0 parts hæmatoxylin ; 90 parts water ; 10 parts alcohol ; 1 part lithium carbonate.

At the end of 24 hours they may be removed, washed in water, and are then ready for the differentiating bath :--borax, 2 parts; ferrocyanide of potash,  $2 \cdot 5$  parts; distilled water, 200 parts.

Some experience and great care are requisite in differentiating the sections, but with patience and judgment thoy can be developed with as much precision as the photographer displays in bringing out tho light and shadows of a dry plate. The sections should be placed in the bath singly, so that each one may be removed as soon as it is differen-The length of time required is variable, depending upon tho tiated. thickness of the sections. The general outlines of the white and grey matter should be known beforehand, and if pathological the seat of the lesion, so that as soon as the contour of the different regions becomes distinct, the sections may be immediately removed from the bath. Over-development destroys the sections, and hence as soon as they are removed from the bath they must be thoroughly washed in water for 12, 18, or 24 hours, to arrest all further development. They are then dehydrated in strong alcohol, cleared in Weigert's clearing mixture :--xylol, 3 parts; carbolic acid, 1 part; sulphate of copper, enough to cover the bottom of the bottle; and mounted in balsam.

The grey matter, connective tissue, and vascular walls are stained light brown; the ganglion cells, dark brown; while the white matter takes on a blackish-blue or purplish-blue tint.

The Pal Method.—This method, which is merely a modification of the Weigert method, was described by Prof. Pal, of Vienna, in 'Wiener Medizinische Jahrbücher,' 1887, p. 589. The preliminary preparation of the sections is the same as with the Weigert method; but with care and dexterity the imbedding in collection may be dispensed with, as they are not rendered so friable and brittle. They are immediately placed in Weigert's staining fluid, to which has previously been added three to five drops of a saturated solution of lithium carbonate for every 10 ccm. of the stain used. After five or six hours they may be removed and carefully washed in water to which some of the carbonate of lithium solution has been added. They are now placed for ten or fifteen seconds in a 1/4 per cent. solution of the permanganate of potash, rinsed in 30 per cent. alcohol, and placed in the differentiating bath:—oxalic acid, 1 part; sulphite of soda, 1 part; distilled water, 200 parts.

The same hints regarding the differentiation of the sections as given under the Woigert method are applicable in this method. Should the sections be very slow in developing, they may be replaced in the permanganate of potash solution and the process continued as before. After they have been sufficiently differentiated they should be carefully washed in water for 24 hours, dehydrated in strong alcohol, cleared in Weigert's clearing mixture, and mounted in balsam.

The medullary nerve-fibres are stained light blue, the neuroglia, connective tissue, and vessel walls are rendered white or yellowishwhite, while the ganglion cells become transparent. To stain these cells, after the development has been arrested by the water-bath, the sections should be placed in a picrocarmine stain for a short time, and the process completed in the ordinary way. To stain the nuclei, the borax-carmine method must be employed as a double stain. These preparations, especially after accepting the double stain, are very pleasing to the eye, and more beautiful than the Weigert specimens. I have also found that they retain their stain much better than Weigert's. A serial section made over two years ago is in as good condition as ever.

The advantages which these two methods offer must be apparent to the most astute observer. To possess a method or methods which will sharply and clearly define for us the limits and boundaries of the white and grey matter of the brain and cord is a desideratum. The differentiation between the two renders the study of pathological lesions very facile, and to follow these changes cephalad or caudad becomes a very easy matter. More especially in tracing separate bundles, or even individual nerve-fibres, have these methods shown their superiority over The Weigert method surpasses the Pal in this respect, all others. being more reliable and trustworthy, and yet with the latter I have traced individual fibres in the pons and medulla with surprising accuracy. Another use to which these specimens may be put is in the study of the changes which the white and grey matter undergo in the transition between cord, medulla, pons, and crura. With the aid of a magie lantern the topography of these regions can be intelligibly demonstrated to a large class, whereas formerly these parts were almost totally ignored on account of their complex and complicated structure.

For the study of angio-pathological and gauglionic changes I prefer the carmine stains to the Pal and Weigert."

Reference Tables for Microscopical Work.\*—In his fourth communication Prof. A. B. Aubert deals with anilin staining.

Alum eosin:—Eosin, 1 part; alum, 1 part; alcohol, 200 parts. Reagent for hæmoglobin. Specimens previously treated with osmic acid 1/2 per cent, for three minutes. Wash thoroughly before staining.

Anilin blue (water solution) :- Anilin blue, 0.02 grm.; water, 25 ccm.; alcohol, 25 to 30 drops. Specimens hardened in alcohol.

Anilin black:—Anilin black, 0.5 grm.; alcohol, 99 ccm.; water, 1-2 ccm. Stains in a few minutes; for brain, &c.

Bismarck brown :---(1) Concentrated aqueous solution, warm, or weak alcoholic solution. (2) Bismarck brown, 1 part; water, 3000 to 5000 parts. For protoplasm, connective tissue, bacteria, living organisms, &c. Material to be hardened in alcohol or chromic acid; wash in absolute alcohol. Mount in glycerin or balsam.

Borax methylin blue:—Concentrated aqueous solution of blue, 24 vols.; borax solution, 5 per cent., 16 vols.; water, 40 vols. Dissolve, filter after 24 hours.

Chinolin blue:—Aqueous solution, 1-100,000 to 1-500,000. For living organisms (water analysis), &c.

Dahlia or Hoffman's violet :-Glacial acetic acid, 12.5 ccm.; absolute alcohol, 50 ccm.; water, 100 ccm.; dahlia nearly to saturation. For axis-cylinder of nerves, protoplasm, nucleus. Stains in 12 hours or less.

Eosin :-- Water solution, or water solution and one-third of alcohol; or eosin, 1 part; water, 1000 to 1500 parts. For epithelium, musele,

\* The Microscope, xi. (1891) pp. 270-2.

axis-cylinder, amyloid degeneration, nucleus, &c. Stains in 1/2 to 1 minute.

Gentian violet: — Filtered 3 per cent. anilin solution in water; conceutrated gentiau violet solution in alcohol; or gentian violet,  $2 \cdot 00$ ; ammonia,  $0 \cdot 5$ ; absolute alcohol, 10. For bacteria, &c. At ordinary temperature stains in about 24 hours; 1 hour at 50° C. Treat objects with 30 per ceut. hydrochloric acid, dehydrate iu absolute alcohol, clear in oil of cloves, mount in balsam.

Iodine greeu :—Iodine green, 0.1 part; water or alcohol, 35 parts. Stains in a moment. Mount iu balsam.

Fuchsin (rosanilin):--Fuchsin, 0.25 grm.; alcohol, 20 ccm.; water, 20 ccm. For nucleus, protoplasm, axis-cylinder, elastic tissue, retina, &c.; after staining, treat with alcohol.

Fuchsin (acid): — Concentrated solution in water. For nervous system. Sections hardened in chromic salts. Keep iu stain one hour, wash with water, put into alcoholic solution of potash (potash, 1 grm.; alcohol, 100 ccm.; filter after 24 hours; use 10 ccm. diluted with 100 ccm of alcohol); wash in water, dehydrate iu alcohol (saturated with salt), mount in balsam.

Methylin blue :—(1) Erlich's concentrated water solution. (2) Koch's concentrated alcoholic solution: methyl blue, 10; caustic potash (10 per cent. 0.2; water 200). 1 and 2 for bacteria, cover-glass preparations; stains iu 1/2 to 24 hours; wash iu water, dry, mount in balsam. For tubercle bacillus; after staining in blue, transfer cover to concentrated solution of vesuvin (15 minutes), wash well in water, dehydrate iu alcohol, clear in oil of cloves (micrococcus brown, bacillus blue.)

Methyl violet :--Methyl violet conceutrated alcoholic solution, 11 ccm.; absolute alcohol, 10 ccm.; anilin water, 100 ccm. For bacteria, &c.; cover-glass preparations; stains in 24 hours; put cover for a few seconds in nitric acid and 3 parts water; wash with alcohol; stain with diluted vesuvin solution for a few minutes, wash in 60 per cent. alcohol, dehydrate in absolute alcohol, clcar in oil of cedar, mouut in balsam.

Methyl green: Water solution,  $2\frac{1}{2}$  per cent. Nucleus, nerves, amyloid substance (degenerated tissue violet, normal green.) Stain in 24 hours.

Safranin :—(1) Safranin, 1 part; absolute alcohol, 100 parts; water, 200 parts. (2) Water, 1 part; alcohol, 1 part; safranin, as much as will dissolve. For nucleus; washed section stains in a few minutes; wash aud dehydrate in absolute alcohol, mount in dammar or balsam. Water solution (1-1200) for bone development (bone, connective tissue, red; cartilage, yellow). Wash with water slightly acid with acetic acid.

#### (5) Mounting, including Slides, Preservative Fluids, &c.

Balsam Mounting.\*—Miss V. A. Latham writes :—"In reading through the January number of 'The Microscope 'I noticed the article on mounting. Cajeput oil is not new to me, as I have used it for several years, also oil of bergamot and several other oils; the last which I am now working on is terpenol or terbenol (Merck). The reason I first

\* The Microscope, xi. (1891) pp. 281-3.

tried the oil of cajeput was the difficulty to mount neatly and evenly sections of human skin and tracheæ, for they curl up when placed in alcohol. This oil is better than clove oil for this purpose, but unfortunately alcohol has to be used, though the process is simplified by using it diluted. I hardly know of the specimens for which I have been compelled to use absolute alcohol, the ordinary spirit being sufficient. In England where methylated spirit is the chief dehydrator used on account of cheapness, then you may require to use absolute The only cases in which I have used the last have been alcohol. where I was making cover-glass specimens of bacteria, and was in a hurry for them to dry so that I could mount at once. Neither is it, I believe, used in the University of Michigan in the histological department. I find my mounts perfect, even those stained with anilins after some four years, and in a paper written in one of the journals of microscopy I casually mentioned it with some other clarifying agents. Turpentine and creosote are used by many to get over the difficulty of strong alcohol; these are very good, but personally I object to the penetrating odours. I would like to say that I think a great deal of difficulty is made over mounting in balsam, which in reality never or seldom exists. True, each method is not nearly so difficult on practically demonstrating as by reading. I give you the method I have always employed for myself and also for teaching, with success so far. Have a mounting card made so that you can use it to centre the slip. In the centre of the slide place a medium sized drop (the second which falls off the rod is about the size); carefully spread the balsam over the surface not quite to the edge of the cover (when it may have been placed in position). Lift the object from the clove oil, drain off most of the oil, except in such sections as lung, brain, &c., and transfer it to the slide in such a way that it is in the centre when mounted, and do not draw the lifter beyond the ring, or the medium runs a little outside the cover and makes an untidy mount. See that the sections have no folds, then take a clean cover-glass in the forceps, and near the edge of it let fall a drop of balsam, invert the cover, and place the point of a needle on the slip at about the place where the edge of the cover is to be when mounted; place the edge of the cover-glass against the needle and gently lower it till the drops meet and flow evenly; when the balsam gets to about the middle of the specimen, slowly draw away the forceps almost parallel to the slip and the cover is then in place, with few, if any, air-bubbles under it. Do not press down the cover with a needle or weight, for unless you have a quantity of superfluous balsam it is not necessary; put the slip away in a warm place in a tray or cabinet, perfectly flat, and in drying the balsam contracts and draws down the cover to the specimen. I often ring slides at once with Hollis's glue, even sending them by post 200 to 500 miles without the least harm. The two essentials are to learn the amount and thinness of the balsam, and not to leave too much clove oil on the lifter. Drying of objects in ovens, &c., seems to me a nuisance, except in cases of mounts without pressure, and even herc I do not advocate it. The formula that I use for balsam may be useful, as it is colourless when mounted and easily made; otherwise the Palmer Slide Company's balsam is the whitest that I have bought of late years, and is a quick

drying medium, more so than the one I use. In fact I am anxious to find some solvent which will evaporate faster, and yet, if possible, at the same time, to avoid much contraction. Obtain a white sample of balsam in liquid form, and take by weight in all cases, in a wide-mouthed bottle, balsam, 3 oz.; turpentine, 1 oz.; ehloroform (pure), 1 oz.; gently turn the bottle several times to mix well, and let it stand till free from air-bubbles and is thoroughly mixed. Pour out a small quantity at a time in a one dram bottle, and keep it covered with a cork, with a glass rod drawn to a point passed through it, or take a piece of glass rod and heat till soft, and press tho two ends together so as to produce a flange. It is then easily wiped and cleaned with a rag moistened in turpentine. Care is taken to lower the cover very slowly. As a question, may I ask if any one who has been to Europe and returned here with specimens, has noticed how tho elimate causes great shrinkage to balsam mounts? As an instance, I have mounts of Cole's studies, and special mounts of his, and I find now that the zinc white has let air pass through and ruined the mounts, in many cases I have had to remount them. I may say here that I am not in favour of the usual white zinc comment, and recommend either brown varnish (Ward's, Manchester, England), or Hollis's glue. The last is the best, for it may be used with immersion lenses, and it renders ringing unnecessary afterwards, which is a bugbear if you should be in a hurry to examine a specimen, which is often the case in medical work."

Mounting Arranged Slides.\*—Mr. G. H. Bryan remarks :—"I havo recently discovered another use for the 'pressureless mounting clips' which I described in the 'Journal of Microscopy' for Jan. 1890. I find, namely, that by their use it is possible to mount slides containing an arranged group of various objects, such as several plant sections or parts of an insect, with great case. To do this, the objects are placed as nearly as possible in the required position in a drop of liquid balsam. On putting down the cover, it will generally happen that, even with tho utmost care, some of the objects will become displaced. Let the cover be now fixed between the jaws of two of my "pressureless clips." Then, by using several badger-hairs or bristles mounted in handles, and of sufficient length to be pushed right under the cover, the objects can be moved about in the balsam and brought into any required positions.

If the specimens again become displaced during the process of hardening the balsam, the slide may be warmed and the objects pushed again by means of a hair thrust underneath the cover into the softened balsam."

Simple Method of fixing Paraffin Sections to the Slide.<sup>†</sup>—Dr. G. Lovell Gulland writes:—"In Dr. Gaskell's interesting paper 'On the Origin of Vertebrates from a Crustacean-like Ancestor,' <sup>‡</sup> he describes a method (on p. 382) by which he succeeded in overcoming the folding of sections of the cranium of Ammocoetes. It consists in floating the series of sections on the surface of warm water, which flattens them, and

1892.

<sup>\*</sup> Internat. Journ. Micr. and Nat. Sci., iii. (1891) p. 328.

<sup>†</sup> Journ. Anat. and Physiol., xxvi. (1891) pp. 56-60.

<sup>‡</sup> Quart. Journ. Micr. Sci., xxxi. (1890).

then transferring them to the slide, which has previously been coated with albumen and glycerin. The sections are then dried by pressure between blotting-paper, the wax is melted, removed by xylol, and the sections are then monnted in Canada balsam.

For some time before Dr. Gaskell published this method I had been using it, and had experimented with several modifications of it; one of these has been so successful in my hands, and in those of others to whom I have communicated it in this laboratory and other laboratories in Edinburgh, that I am desirous that it should become more widely known.

In using albumen and glycerin as a fixative, according to Meyer's method, which is, I suppose, the way in which Dr. Gaskell employs them, I have met with objections which made me wish to omit that part of the process. These difficulties are :- That it is not always easy to get the layer of albumen and glycerin of equal thickness all over the slide, so that the sections do not lie quite flat; that patches of coagulated albumen sometimes retain stains, cspecially some of the anilins, in a disturbing manner, and this inconvenience, I am told, is specially felt in microphotography; that in manipulating sections on the slide, solutions of pierie acid, moderately strong alkaline solutions, and some other fluids eannot be used, as they loosen the adhesion of the section to the slide. Further, I have found, in using Dr. Gaskell's method, that if a very large number of sections are to be mounted on one slide, the fixativo is apt to be washed off; and if one trusts to the fixative afterwards, and proceeds in the usual way as described by him, some of the sections may be lost. The method I use is the following :----

The piece of tissue is imbedded in paraffin in the usual way, and I will suppose that a complete series of sections is desired, and is to be cut with the Cambridgo rocking microtome. The paraffin block containing the tissue must be trimmed very carefully, care being taken to see that the surface meeting the razor is exactly parallel to the opposite surface, and that the block is exactly rectangular. A thin layer of soft paraffin is then applied to the surface meeting the razor and to the opposite surface-this is best done by dipping these surfaces into the melted soft paraffin-and when this has become firm the surfaces are again trimmed square. The reason for this very special care is that any curve in the ribbon, produced by neglect of this precaution, is accentuated by the flattening out of the sections, and though in mounting several ribbons on one slide a slight curve does not matter, and can, indeed, be corrected by folding up the soft paraffin between the sections, a sharper curvo of course interferes with the regular disposition of the scries. When all the sections required have been cut, the ribbon must be divided with a sharp knife into lengths corresponding to that of the slide in use. A very convenient size is a slide of 4 by 2 in. with a cover-glass of 3 by  $1\frac{1}{2}$  in. These ribbons are then to be seized at one end with forceps, and the other end is gently lowered on to the surface of the warm water, and as the sections flatten out they will be found to move along the surface of the water, so that more and more of the ribbon can be lowered. It is not so satisfactory to hold both ends of the ribbon and lower the middlo first. When the flattening is complete, the slide, carefully cleaned, is immersed in the water, the ribbon is floated

into its position on the slide with a stiff brush, and the process is repeated with one ribbon after another until the slide is full. With a little practice one soon learns how to bring the rows elose together so that no space is wasted. The slide is then set up on end to allow the superfluous water to drain off.

The best dish for carrying out this process is, perhaps, a flat glass dish standing on a dark table, as the manipulation is more easily accomplished when the white paraffin is thus thrown up in relief. The temperature of the water is of eourse important, but as different workers use paraffins of varying hardness, no absolute rule can be laid down. It should be comfortably warm to the hand, but never so warm as to melt the soft paraffin holding the sections together. Short of this, however, the warmer the water the more rapidly and completely are the sections flattened.

So far the sections are simply lying loose upon the slide, and they have yet to be fixed to it. This is done by evaporating the water from the surface of the slide. The evaporation might be carried out in many ways, but I shall best explain it by describing my own practice. I almost invariably use paraffin for imbedding whose melting-point is 52° C., and the imbedding oven, an ordinary copper one, is therefore kept at about 54° C. or 55° C. The slides, after the water has drained off as much as possible, arc placed on the top of the oven, where the temperature is probably a little under  $50^{\circ}$  C., and where, eonsequently, the paraffin of the sections is not melted, though the water rapidly evaporates. The slides are kept there, with a cardboard cover over them to keep off dust, until the evaporation is complete, and the sections have adhered to the slide. The time required for this varies, as I shall show immediately; but the important point is that the paraffin must never be melted until the last trace of water has disappeared from the slide. If this premature melting happens by any accident, the sections are certain to peel off later. When the water has evaporated completely, the opacity of the sections disappears, they become much more transparent, and they look dry. A very few experiments enable one to be sure of the point when slides are safe. Of course when the paraffin used for imbedding is of a lower melting-point than 52° C., the temperature for evaporation must also be lower; and when the oven is regulated as above, this can be managed by putting a few thicknesses of paper under the slide.

When the fixation is complete, the paraffin is melted by putting the slide inside the oven for a little, and is then washed off with turpentine or xylol; and, if the piece of tissue has been stained *en bloc*, the sections ean be mounted at once in balsam.

One of the great advantages of this method is the perfect ease and safety with which it allows sections on the slide to be manipulated, so that the most various stains and reagents can be applied successively to a slide, e. g. the complicated processes used to demonstrate bacteria in the tissues can be applied, with the certainty, moreover, that there is nothing on the slide to be stained which was not in the section.

The time required for complete fixation varies in dependence on several circumstances, but of these the most important are the thoroughness with which the superfluous water has been drained off the slide, and the thickness of the sections. For instanco, sections cut with five teeth of the rocking microtome require generally about an hour to dry in the way I have described; those cut with ten teeth perhaps three hours; while those cut with fifteen teeth take six hours, or even longer. This scale is only approximate, and it may be said, generally speaking, that the longer the slide is allowed to dry the better will be the fixation, and, of course, no harm is done to the section by leaving it for an indefinite time in paraffin, so long as the paraffin is not melted.

Of course a single section is to be mounted in the same way as a series, and it will be found that where it is desirable to examine a few sections with as little delay as possible, warm methylated spirit, or even absolute alcohol, evaporate more rapidly than water, while the fixation is as perfect with them, and the method of use exactly the same, as with the less volatile liquid. For obvious reasons these fluids are not likely to be used frequently with long series of sections."

### (6) Miscellaneous.

Detection of Adulteration in Linseed and in Linseed-oil Cake.<sup>\*</sup>— M. J. Van den Berghe finds that linseed oil-cake is adulterated in commerce by a large number of foreign substances, among the most frequent being colza, mustard of various kinds, hempseed, *Ricinus*, *Arachis*, poppy, &c. For detecting these adulterations he recommends treating the linseed successively with sulphuric acid  $(2 \cdot 5 \text{ per cent.})$ , soda  $(2 \cdot 5 \text{ per cent.})$ , alcohol, and ether, and then digesting for some hours in the cold with a concentrated solution of calcium chloride. This makes both the pericarps and the testa of seeds so transparent that the distinctive characters of the various kinds can be readily recognized under the Microscope. The nutritive reserve-substances of linsced being chiefly aleurone-grains and drops of oil, iodine solution should not give the blue reaction when the oil-cako is pure.

\* Tourteaux et farines de lin (6 pls. and 24 microphot.). See Bull. Soc. Belg. Micr., 1891, p. 160.

164

# MICROSCOPY.

#### a. Instruments, Accessories, &c.\*

#### (2) Eye-pieces and Objectives.

Microscope Objectives.<sup>†</sup>-Prof. T. J. Burrill read before the American Society of Microscopists the following paper:-" I had the honour of presenting to this Society, at its last meeting, a paper embodying my experience and opinions concerning the Microscope. I now wish to offer the result of personal experience in the use of various objectives for microscopical work, especially along the lines followed as a teacher and investigator of biological science. The task thus set before me is more difficult than that of last year. Little niceties of difference count much more in an objective than in the construction of stage, or rack-andpinion adjustment; and though one may be sure that his preference is not founded upon fancy, yet he may find it hard to state in words upon just what special characteristics he bases his choice. In the paper of last year the names of makers are carefully excluded; this time it is impossible to get along without reference by name to the manufacturers of the instruments cited. I heartily wish it could be avoided and accomplish the purpose intended, for it is a source of embarrassment to myself, and is also liable to be seriously misin-All that can be said in justification of what follows, is terpreted. that I am under obligations to no one, either directly or by implication, except as necessitated by truth and fair dealing, and that matters of personal interest are thoroughly placed aside, it I am capable of so doing. The articles used are all owned by myself, or by the institution in whose service I am, with one somewhat conspicuous exception, and that was loaned to me, upon request, for the purposes of this paper. No comparison is made with such as I have not had abundant opportunity to test, and, with the exception just mentioned, with nonc that have not been in use during some years of time. In the paper upon Stands, a note was made upon the fact that we are prone to like best that with which we become acquainted. In the case of objectives, however, there is less room for such preference, because the mere handling of one is practically that of others, including the position and movements of one's body when at work. To be sure, in order to get the very best results with a high quality objective, one must patiently learn to use that particular instrument; but this is another The force of habit has little to do in this last case, while it is thing. exceedingly strong in the method of moving the object under the lens, and in the manipulations generally of the stand.

It should also be stated that my work has chiefly been upon uncoloured objects mounted in water, with or without the addition of carbolic acid or glycerin, and upon coloured objects in balsam; the main exception is that of diatoms in balsam, and in this case as a test for the objective rather than work upon the objects for their own sake.

<sup>\*</sup> This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous. † Microscope, xi. (1891) pp. 321-8.

# 256 SUMMARY OF CURRENT RESEARCHES RELATING TO

Magnification.—Whatever may be the facts in regard to the use of high-power eye-pieces to secure the requisite magnification in mere tests, for long-continued work over the tube anything in the upper end of less than about one inch focal length is unsatisfactory to me. The strain upon the eye is certainly less with the medium and low-power oculars, and the image is better to my eye, even with the finest objectives made. I chose, therefore, such focal length in the objective as will give sufficient magnification with a Huyghenian eye piece, amplifying about ten times as the upper limit. Higher magnification by the eye-piecc may be useful in testing an objective, and may, it is true, to some persons, be available for long-continued work; but I am making a report of personal experience. The only other thing necessary to say here is that usually the less amplification the better, after a suitable amount is obtained. Hence neither objective nor cyc-piece should be of less focal length than will conveniently serve the purpose required. For a botanical laboratory a 1/2 in. and a 1/5 in. dry objective are the best selection for the common work of students. Occasionally higher powers are needed, sometimes running up to the highest and best procurable. For these exceptional cases provision should be made by having a few objectives at hand, but students need not be furnished with them as with those first named. Really serviceable magnification seems to reach its limit in about a 1/15 in. or at most a 1/18 in. objective. Only in rare cases is anything of higher power than a 1/10 in. of best quality effectively superscded-with me, in nothing but certain studies upon bacteria.

Angle of Aperture.-It appears to me that something similar can be said of the angle of aperture. In the matter of difficult resolution with oblique light, high class and even medium grade objectives have been, in my hands, proportionally successful in just about the order of their aperture, though exceptions have been noted. But for most other uses, it does not appear that the angle of aperture should be relatively rated so high, in the qualities of objectives. It must not be inferred from this that wide angles are, in and of themselves, injurious for biological work. Other things being equal, I should always prefer them, cheerfully putting up with any lack of penetration, and, to a certain extent with inconvenient working distance, for the other advantages offered; but crispness of outline, of even the smallest bacteria, depends upon something else quite as much as upon the aperture and cost price of an objective. These smallest bacteria measure about 1/50,000 in., or about the distance apart of the dots from centre to centre of Pleurosigma We all know that great angle is not necessary upon objects angulatum. The question is whether excess of angle above a certain of this size. essential degree is of any importance whatever, or indeed, whether an objective of wide aperture is, on this account, especially superior, when the illumination is a narrow beam of axial light. When the object is too small or too slender to be seen by a narrower angle, no doubt can exist even in this case of the essential advantage of the greater aperture, but unless one wishes to see the flagellum of a bacillus, or a minute structure of a diatom valve, his laboratory work may, perhaps, be just as successful with first-class objectives, of less than the widest angle procurable. Should these of moderate angle possess better definition (not resolution), then for their proper work they are better lenses. My later purchases, for students' ordinary use, have been of  $110^{\circ}$  air angle for 1/5in., with the expectation that anything up to the widest numerical aperture may sometimes be accessible. For the closest possible studies upon the exact size (measurement) and shape of small stained bacteria, a Tolles' 1/15 in. homogeneous immersion of  $123^{\circ}$  balsam angle is the best I have used, though others at hand have considerably wider aperture.

Get the best.-Having decided what is most suitable for the work proposed, the very best should be selected for students' use, as well as for special investigators. It may be said that the expense would often be too great, and that cheap instruments or none constitute the alternative. Often, however, this is the mere outgrowth of too cheap ideas, either on the part of the instructors or boards of trustees. If the real needs are fairly appreciated, in this as in any other case, they can usually be met in some way. Otherwise, how are Microscopes obtained at all? At any rate, instructors should inform themselves with the utmost care and then equip their pupils in the best possible manner with this, the most delicate of all tools. No questions of home or foreign manufacture, of accidents of popular approval or of hereditary service, should be allowed weight in the selection of a Microscope objective. Neither should the cost price be taken as an index of quality. No one can be blamed for buying what he finds to be the best goods for the least money.

Governed by these principles, I have ceased ordering from abroad, for students' use. Without naming other makers, I choose the objectives of the Bausch and Lomb Optical Company, in preference to those of Leitz. I have in daily use some first-class wide-angle dry objectives of the Gundlach Optical Company, that have given most excellent satisfaction. Anxious to have the best, as improvements were announced, I have ordered, from time to time, five first-class objectives, each one supposed at the time to be the very best in the market. This paper may seem less presumptuous with this statement inserted.

Specific Tests.—I am now to report the results of some comparative tests, made with certain named objectives, under described methods of procedure. When the title of this paper was announced I hoped to have photographs taken in different ways for each objective tried, but have found too much time consumed in other directions to permit it. Please allow me to express the conviction, that these proposed photographs would have certainly corroborated the statements herein made.

In order to decide, with certain correctness, of the relative quality of the objectives compared, the tests were purposely made as difficult as circumstances permitted, but under these difficulties each was given the best handling possible for the manipulator. The objectives, all homogeneous immersions, were as follows:—Tolles' 1/15 [1880] 123°; Zeiss' apochromatic 1/12 [1887] N.A. 1·40; Herbert Spencer's 1/10 [1888] balsam angle 130°; Gundlach's 1/12 [1890] balsam angle 136°. The last was asked for and loaned to me for trial. An attempt was also made to include a Leitz 1/12 [1888] N.A. 1·25, but it was not possible to use it on the same stand, hence not certainly under the same conditions, and not included in this report. I have not been able, under fairly similar conditions, to make it do what is reported for the others.

The first tests were made upon Möller's balsam-mounted test-plate, with an ordinary small coal-oil lamp, with flat wick 3/4 in. wide, and common round chimney, on which, however, was placed a tin extension 16 in. long, to improve the combustion and steady the flame. Any one who sits down to a prolonged task of this kind will appreciate the latter, at least, of the improvements thus obtained. The lamp was placed 30 in. from the mirror to the left, with the centre of the flame used edgewise, and mirror of the same height. The mirror-bar was placed at the angle of 51° from axis of the instrument, and the eoneave side accurately focused by means of the paper label on the test-slide. No substage helps of any kind were used. After adjustment of objective and light as described, an ordinary bull's-eye, same height as flame or mirror, was pushed in and out at will near the lamp, flat surface to the latter. The tube of the instrument could be closed to 61 in., measured from its lower end to top of draw-tube, and could be elongated to 13 in. The Tolles' and Spencer's objectives have screw collars; these were adjusted for their best effect with tube-length of 10 in., measured from front of objective. For the others the tube was varied to suit. It should be said that the Zeiss objective was ordered for the long tube.

Amphipleura pellucida, on this particular slide, is of medium grade as to difficulty of resolution, but as difficult as any I have seen in Möller's test-slides. No. 19 is probably proportionally easier, often showing by light and adjustments which No. 18 defies. I think this last is unusually difficult, and the same may be said of No. 12, Grammatophora subtilissima. The others seem to be fair average shells. As immersion media, somewhat thickened eedar oil, as furnished by Zeiss, and a fluid sent out by the Gundlach Optical Company, were used successfully with all the objectives, with, however, no perceptible difference in result. The work was done in the daytime, with windows behind the operator, uncurtained. There were no windows in front or at the sides.

Under these conditions all four objectives resolved Amphipleura so plainly that any tyro could make out the transverse lines, at least, when the bull's-eye aided the illumination. Often the lines appeared the moment the focus was secured, and this could be changed back and forth with almost certainty that they would be evident whenever the proper adjustment was made. I need not say, however, that it always required earcful work, and that there were failures as well as triumphs.

The two non-adjustable objectives did best with the shortest tube and negative ocular. With Zeiss's compensating ocular the result was rather more satisfactory with the 10-in. tube-length. There did not appear to be the same difference with the Gundlach in this respect, the Zeiss eyepiece also showing well with the short tube. With the apochromatic at its best, the diatom appeared perfectly flat, with midrib and margins showing distinct and clear, when the lines were in focus—a thing none of the others did, though Spencer's, perhaps, came nearest to it at 8, with 10-in. tube. The whole field, too, of the first named, including the object, was beautifully white. With the Gundlach it seems to me that the lines were as distinct and crisp as with the Zeiss, and could be counted with reliability a few at a time. When these were best shown the raphe
and margins glowed with rcd, shading to dark, and a little movement of the focus downward was necessary to render the margins most distinct. With a longer tube the lines more evidently stood above the outline. With the Spencer, at its best, I found little changes of illumination, &c., destroyed the resolution to a more marked degree than with the two others just named; and though the lines were becautifully shown, and the outline fair at the same time, it seemed to me that counting would be a much more difficult undertaking. It should be remembered, however, that the magnification was less, and this I could not fairly make up with higher eye-piecing. Under a solid 1/4-in. ocular I was unable to make any distinction in the quality of the lines. With all three objectives they were like parallel ropes, with uneven and woolly outlines.

The Tolles' objective gave the ontlines readily enough, but partaking somewhat of the character just described, with the solid ocular. With the magnification reduced to that of the 1/12 by the use of longer eyepieces the haziness of lines partially disappeared, but in no way seemed so beautifully sharp as in the other cases. In both the Spencer and the Tolles there was a tinge of red in the raphe, in some cases merging into a dark shade, when the lines showed best under manipulation of the screw collar.

Upon the whole, it seems to me, the apochromatic, in this special test was really in the lead, though the distinction had to be carcfully drawn. The results on other diatoms on the plate were similar, so far as could be determined, the rating of the objectives remaining the same.

I next tried the mirror in exactly central position, with other things remaining the same, save as the height and position of the lamp and bull's-eye required changing. This was varied, too, during the same test by inserting a narrow-angled 1/5-in. dry objective as a condenser, taking great care that it was in central position. In each case, to be further assured that the illumination was axial, examination was made by removing the ocular and looking at the bright spot in the back lens of the objective. The difference in the performance of the objectives was certainly less marked than with oblique light. The required tubelengths remained about as before stated, with, however, less noticeable difference in a given amount of change. I obtained a kind of a glimmer of resolution on No. 19 with the apochromatic and Gundlach's lenses, but nothing with any of them on 18 or 20. The others were well resolved by all four objectives, Grammatophora subtilissima giving the most I have never seen a balsam-mounted Amphipleura resolved by trouble. truly central illumination, though others have reported it with several objectives. When using the condenser named, by moving it only a little to one side, lines could be made out, but no comparative tests of this kind were made.

I had previously tried, with the help of an expert assistant, the three objectives in my possession in photographing violet-stained bacteria with central light, showing a scarcely appreciable difference, but favouring the Zeiss and Spencer over the Tolles, unless the increased difficulties with the higher power proved too much for the skill of the manipulators.

I have now to add a word in regard to the durability of the apochromatic, the want of which has been frequently questioned. After about two years' use it became that this lens was in some way impaired, and by looking through it from the back with a magnifier a hazy-granular appearance was noticeable, not due to dust on the back lens.

Last March the objective was sent to the makers for examination and repair. It reached me again in July, as good as new, with the statement that the front lens had been slightly decentered, and that the repair had been easily made, and was without charge. I have no other information upon this point, neither do I know what interpretation to place upon the granular appearance noted. There is certainly nothing of the kind visible now."

Fluor-spar Objectives.\*-The following letter, communicated to the American Society of Microscopists, will be read with interest.

Prof. T. J. BURRILL, My dear Sir. -- Your favour † of 21st inst. came to hand this morning. Since coming to Buffalo my time has been so fully occupied that I had for a long time forgotten entirely the subject mentioned in your letter. As the time is so limited, and having no knowledge of how much space you had intended to give to the subject in the forthcoming publication of the 'Proceedings of the A.S.M.,' I have thought best to state to you, as briefly as possible, the facts, leaving it to you to arrange them in proper form for publication, with whatever comments you see fit to make.

During the summer of 1860, Dr. Rufus King Brown, who was at that time a resident of Brooklyn, New York, visited my father at Canastota, and during his stay there my father made for him a 1/8 objective, which was considered by all who saw it to be the best ever made up to that date. Although but a young boy at the time, I was greatly interested in my father's work, and knew pretty well what was going on-hearing a great deal of talk-and remember well Dr. Brown's high praise of the performance of the objective, but of course knew nothing of its construction until some years later. Some years later, after Mr. Tolles had removed his establishment to Boston, Dr. Brown became a resident of that city, and showed the objective to Tolles, who praised its performance very highly.

The angular aperture of the objective was 175°. One of the systems contained fluor-spar, and it is on record in the formula that it was remarkably perfect in its corrections for both figuro and colour, with both oblique and central illumination. In the years 1864 and 1865, I made lenses for quite a number of objectives, mostly 1/4 in., containing at least one lens of fluor-spar, and having apertures from 170° to  $176^{\circ}$ ; but of course with very short working focus. In all these objectives, as well as in the one made for Dr. Brown, the spar lenses were cemented between others, as owing to its softness and liability to become scratched it was not considered safe to leave it in an exposed About the time that immersion lenses came into general position. favour in this country the use of the spar was abandoned, owing to the difficulty experienced in procuring that which was free from fractures

\* Proc. Amer. Soc. Micr., xii. (1891) pp. 248-9.

+ This letter was received by me January 31st, 1891, after the preceding pages were in the press. Prof. Burrill informed me by accompanying letter that he had sent a request to Mr. Spencer for information as to the facts about fluor-spar objectives .--[ED. Proc. A.S.M.]

or seams. When used in the 1/4 I dreaded making them more than all others in the objectives, often having to throw them away, owing to their having such defects. Shortly after our removal from Canastota to Geneva I made a 1/12 water im., containing one spar lens; but that is the only one since I was a boy. When Dr. H. J. Detmers, of Columbus, O., visited me at Cleveland (about the time of the meeting of the A.S.M. at Detroit) I showed to him the old book containing the records of all these objectives as kept by my father, and among them the 1/8 marked R. K. B., also 1/8 containing a spar lens, which was made as far back as 1851, about the time my father began making objectives of large angle, showing how early in the fight he appreciated the valuable optical qualities of fluor spar in the construction of objectives. The angular aperture of this 1/8 was not given, but I ean readily see that it could not have been less than  $160^\circ$ .

These are, I think, all the facts necessary, but I may have made too much of it. It has been hurriedly written off and is not in proper shape for publication, but you can call out the facts and arrange them properly for publication in the forthcoming proceedings. I shall esteem it a great favour if you will do so, as I look upon the facts as of value in connection with the history of the Microscope.

I trust you will pardon the use of the pencil, for I have been anxious to send this by the first mail. Please let me know if it reaches you in time.—Very truly yours, H. R. SPENCER.

Fluids for Immersion Lenses.\* - Dr. A. C. Stokes remarks :-"Usually the only immersion fluids at the microscopist's command are water, cedar oil, and glycerin made dense by dissolving in it either cadmium sulphate, zinc carbolate (sic) or some other salt. With homogeneous-immersion objectives, or those using an immersion medium with a refractive index as nearly as possible that of crown glass, so that the cover, the immersion medium, and the front lens may form one homogeneous combination with these objectives, water of eourse cannot be used; so that the microscopist must have resinated cedar oil or the glycerin solutions just referred to. But to obtain the best results from these first class homogeneous-immersion objectives it is important that the immersion fluid should have the proper refractive index, that of crown glass being 1.5, of eedar oil 1.515, the glyeerin fluids varying in a way that the microseopist has usually no means of finding out. Prof. H. L. Smith has devised a simple and successful little instrument for the measuring of the refractive index of such liquids, but, so far as I know, it is not in the market. The microscopist must therefore rely on the optician, that sometimes by accident plays him false, and so deprives him of the best that his objectives can do. I have recently had an experience with these substances that has taught me, if not wisdom at least caution in blaming my objectives or even my own lack of manipulative skill.

A certain homogeneous-immersion objective, of not large numerical aperture, was said to be able to resolve *Amphipleura pellucida* well and easily. I made the attempt, and failed, after several hours' work with the lens, using all the care and skill that I possessed. The immersion

\* Microscope, xi. (1891) pp. 341-4.

fluid used had been prepared and sold by a prominent optician, and I had no thought but that its refractive index was what it should be. Another evening was given to the examination of the lens over the same diatom; failure. A third evening was devoted to the same work, and failure was the reward. I then gave it up, and condemned the objective or my own skill, being disposed towards lack of confidence in the latter. Yet others had said that that objective would resolve that diatom. A fourth evening was given to it with the same result. Then it suddenly seemed stupid not to think to try another immersion fluid. There might be something lacking in this. I had cedar oil from a well-known European optician, and with a drop of it the objective was focused, with the light as oblique and the mirror exactly as before, when the lines on that shell stood out, if not like the pickets on the fence, at least with a sharpness, clearness, and neatness that was as delightful as it was amazing. In the twinkling of an eye the diatom was resolved to perfection, while with the glycerin fluid, failure and discouragement had been the only results. The objective was vindicated, and so was any skill that the observer might, in a moment of self-complacency, imagine to be his. But on the table were two other glycerin fluids, one by a prominent and accomplished optician of this country, the other by a famous American, who is by all odds the equal of any optician in the world. The immersion fluid from the latter refused to have anything to do with those lines; its action being similar to that of the composition first tried. But the objective was not at fault, nor the adjustment.

The other fluid was then tried, and the resolution was in every respect the equal of that made with cedar oil; if anything it was superior. But there was as usual the fly in the ointment. To remove the glycerin from the objective it is necessary to wash it off with water, but in this case, when the water drop was added, I had a moment of anxiety, for the fluid became white and opaque as milk, and I could see white particles falling on the lens front, like little flakes of snow. Investigation proved that the salt dissolved in the glycerin, a solution which makes so perfect an immersion medium, acts chemically on the nickel plating of the objective, and the glycerin seizing the water, allowed the new salt to fall in opaque white particles. The chemical action is so great, that after using the medium for three times, there was deposited on the cover of the test-plate an iridescent film, having an irregularly circular outline, showing where the metal and the fluid had been in contact. Nor is this all, for across the surface of the front lens itself is a streak of the same insoluble iridescent deposit. The optician declines to make known the composition of the fluid, although he might reveal it with confidence, since no microscopist would ever make the medium for his own use after having a little experience with it. Its action on brass is similar to that on nickel, and must forbid its use as an immersion medium, although it is really the equal of the renowned cedar oil. To the latter, useful as it is, valid objections are its tendency to flow too freely, and the trouble needed to clean it from the lens, alcohol being demanded to remove it entirely, whereas with glycerin, a drop of water is enough. Cannot some of our opticians give us a glycerin medium

262

with the refractive index of the resinated cedar oil, but without the obnoxious quality of the fluid that acts on the objective mounting? For these learned men the problem should be an easy one. The maker of the dangerous glycerin mixture can surely make something as good. I hope he will never make anything quite so bad, although in its optical action it is as nearly perfect as need be wished. Its hunger for metal is the fatal objection to it.

Upon the optical action of the immersion fluid depends the optical action of the homogeneous-immersion objective. If the former is not of the proper index, the microscopist may deceive himself by believing that his objectives are giving him the best possible results; or if they seem to be optically defective he should remember that the fault may be in the fluid supplied by the dealer. The optician should place at the disposal of every microscopist some simple device by which the refractive index of the immersion medium may be ascertained. Zeiss sends out for this purpose a wedge of glass, which, when used as directed, gives the desired information. Prof. H. L. Smith's device is not obtainable. and that of the German optician can be had, I suppose, only by buying one of his homogeneous-immersion objectives. Without some such means, the microscopist can never know whether he is getting the best work from the objective or not, unless he attempt to resolve the proper diatom every time he begins to use a fresh supply of immersion medium, a method that would be time-consuming, and should be unnecessary. While with the improper fluid he may get moderately good results, with a medium with the correct refractive index he will get the best that the objective can give, provided of course the lens be properly manipulated."

## (3) Illuminating and other Apparatus.

A new Modification of the Abbe Drawing Apparatus.\*—Dr. W. Bernhard deprecates the discredit into which microscopic drawing has fallen, owing to the enormous advances recently made in photomicrography. Without denying the immense practical advantages of photography, he considers that there are many cases in which the objectivity of the photograph is not desirable. In microscopie investigations we often require to know what the observer really saw, not what he could have seen, and it is only a drawing which can give expression to such subjective observations.

The cause of the complaints brought against drawing apparatus on the ground of indistinctness of the image and of the point of the pencil is not due to any defects in the optical parts of the apparatus, but mainly to a want of proper regulation of the light. All drawing apparatus havo this in common, that the plane of the image is projected upon the plano of the drawing. With unequal intensities of the light of the two surfaces, it is clear that the more intense will have the effect of making the less indistinct. In order to see clearly at the same time the plane of the drawing and point of the pencil on the one hand, and the Microscope image on the other, the intensity of the light must be made the same for each. This is effected by reducing the intensity of the light either of the image or of the plane of the drawing.

\* Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 291-5.

In the Abbe drawing apparatus two smoked glasses are provided for reducing the light of the plane of the drawing, which is usually the more intense. In order to allow of finer gradations in the reduction of the intensity of the light of both image and drawing plane, the author has modified the Abbe apparatus in the following way:—For the two smoked glasses for diminishing the intensity of the light of the drawing plane are substituted two circular diaphragms, each of which contains four smoked glasses of different degrees of darkness. These are hinged on an arm so that each alone or both together can be inserted between the prism and mirror, or rotated to one side at will. The central position for each glass between the prism and mirror is marked by a catch.

For reducing the intensity of the image, a third horizontal rotating diaphragm is placed below the prism; it is of the same size as the other two, and, like them, possesses four apertures, three of which contain smoked glasses, while the fourth gives free passage to the rays out of the Microscope. The three diaphragms together allow of exactly 100 possible combinations.

Winkel's new Drawing Apparatus.\*-Dr. H. Henking describes the new drawing apparatus of Winkel. In principle it is not new, since it closely resembles the apparatus described by G. Kohl, as well as that recently brought out by the firm of Zeiss. In all three the image is projected by a mirror inclined at 45° upon the horizontal table on which the Mieroscope stands. The union of the plane of the object and plane of the image on the retina is effected by Winkel by means of a prism, which carries in the centre of the hypothenuse face a small glass eylinder. This eylinder is eut obliquely where it is attached to the prism, so that it stands vertically and transmits to the eye the rays coming from the Microscope. The hypothenuse face of the prism is silvered. For diminishing the intensity of the light he inserts between prism and mirror a rotating diaphragm having five apertures, three of which contain smoked glasses, while the fourth carries a bluish glass, and the fifth has none. The correct position for each glass between prism and mirror is marked by a eatch. The arm which earries the mirror is in two parts, so that the distance of the mirror can be varied by one part sliding in the other. Prism, rotating diaphragm, and mirror are earried on a common arm, which is not in rigid connection with the spring socket by which the apparatus is attached to the Microscope, but is movable about a horizontal pin, so that the arm with prism, &e., ean be rotated to one side. Lastly, a special clamping-serew permits of the adjustment of the height of the pin, and with it the prism, &e., so as to suit different eye-pieces.

The Grapho-Prism and its Use.<sup>†</sup>—Dr. F. Gaertner writes :—" To a practical microscopist who is not also a skilled artist, perhaps nothing is more important among microscopical accessories than the camera lucida, or grapho-prism. This prism is an instrument for sketching objects with the point of a peneil upon a piece of paper laid beside the Microscope. By its use a high degree of accuracy may be attained. Perhaps the simplest and most successful drawing prism is that of Zeiss, which is

\* Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 225-7,

† Amer. Mon. Micr. Journ., xii. (1891) pp. 265-7.

followed by that of Nachet, Abbe, and Oberhauser. Nobert's and many others work upon the same principles.

The following is an explanation of the principle of this drawing apparatus:—If the glass plate gl (fig. 29) stands at an angle of  $45^{\circ}$ with the axis of the eyc, the rays from the object o (which on their part also form an angle of  $45^{\circ}$  with the glass plate) are reflected, and the picture of the object is seen in a position that also forms a right angle with that of the object. If m is the cylinder of the Micro-



scope, and pp the piece of paper, in this case the eye will see upon the paper at o the picture which is projected by the transparent condition of the glass plate gl. In this case we say that the picture is projected; but if we place a prism p (fig. 30) upon the same level with the glass plate gl, and o is the object under the Microscope standing in a vertical position m, the glass gl forming an angle of  $45^{\circ}$  with the axis of the eye and standing upright over the ocular, we will then see the picture at o' upon pp. Meanwhile, the projected picture of the object may also be seen in the horizon. Upon this basis rests the abovementioned drawing prism, of which Nachet's is the one most commonly in use in Europe after Zeiss's. In this apparatus a prism is employed in place of the glass plate, while a second grapho-prism moves upon its own axis so as to bring the reflecting surfaces at different angles. The purpose of a drawing prism is obvious as soon as it has been placed upon the ocular and adjusted.

Oberhauser's camera is somewhat more complicated than the others. For this reason I will give more in particular the details in regard to its practical application. The ascending rays from the objective are totally reflected through the large prism d into the horizontal arm A. If the ocular is placed in a horizontal position B it directs the rays into the small prism C upon the figure of an angle of  $45^{\circ}$  if focused in the right position, where it is again reflected at a right angle into the observing eye.

Oberhauser's camera is very much liked for this reason: it does not create a disturbance or a confusion by the reflection of the picture at a right angle upon the projected paper placed in a horizontal position. The Oberhauser camera is attached to the tube of the Microscope at the ocular end, without any trouble or loss of time. With but one exception this camera is perfect; it has a deficiency in one particular. When the microscopic picture is twice reflected it then loses considerably in accuracy, that is, in its clearness and exactness. This is especially so in the use of higher powers, oil-immersions, &c. By the most concentrated light only can the special and superficial contour of the microscopic picture be best produced.

The practical and most applied drawing apparatus in microscopical work is the camera lucida. The object and the paper are seen with the one eye, and at the same time the picture is reflected into the eye by means of the mirror or prism. As the picture is seen upon the paper beside the Microscope, its contour can be reproduced upon the paper with the point of a pencil, and that too with mathematical and scientific exactness; but he who has by practice learned to look into the Microscope with one eye and to hold the other eye open at the same time, may get along even without the use of a camera lucida if he gazes with one eye into the Microscope. In a few moments the observer will find the object projected upon the paper and will thus be able to sketch the outlines with comparative ease and exactness.

In the execution of the drawing of the microscopic object it is best to use strong paper-Bristol paper or Bristol board-and the paper should be either pale-yellow, pale-green, or white and slightly shaded. It is also advisable to have the paper fastened upon a smooth board. First use a soft and finely sharpened black pencil in order to secure the outlines and the contour of the picture. It should be slightly shaded, without pressure. Then, with bread-crumbs, rub most of it out again. After that, with a heavy pencil, retrace the outlines of the first drawing again, using the prism for comparison and exactness. This moment is the proper time to do the shading, if such is required, and this can easily be done with the point of a pencil and a rubber, or, still better, with charcoal and soft cloth. For drawing a picture with colours, water colours are most commonly used; after them coloured lead pencils, oil colours, and pastel crayon. I wish here to call special attention to this fact, that in shading it is advisable to shade off the uncoloured parts first with black; particular care must be taken that the shading does not extend into the coloured field. It is also decidedly recommended to use a variety of colours, especially in the drawing of very minute objects such as endothelium and epithelium cells, fibrous and connective tissue cells, blood and lymphoid cells. In drawing a whole slide, or only a part of it, it is sometimes desirable to use a variety of colours. Not only will it make a drawing more elaborate, but decidedly more comprehensive and instructive.

Virchow, the most expert pathologist of the nineteenth century, has said that he would not give "ein Pfennig" for illustrations, drawings, or sketches that were not correct and exact, because in every instance it would convey a false impression. Besides this, Virchow has said that all lectures, demonstrations, original articles of any kind, should be accompanied by first-class drawings or illustrations.

266

I, therefore, would advise every practical and expert microscopist, especially microscopists that are not artists in drawing, sketching, and in the art of producing microscopic illustrations, to make use of the grapho-prism. Especially so would I advise students of practical histology, physiology, pathology, pathological anatomy, bacteriology, embryology, and pharmaeology to use the grapho-prism hand in hand with the Microscope."

A new Mechanical Stage.—The following is the text of the remarks made by Mr. H. Bernard when exhibiting his new stage at our meeting on December 16th, 1891:—

The disadvantages of the mechanical stages hitherto in use in the microscopical world are too well known to need mention here. It is enough to say that they fail one exactly when they are most needed, viz. in the systematic study of large objects, such as series of sections, culture-plates, zoophyte-troughs, &c.

The principle of the new stage is such that it is capable of adaptation to almost all the requirements of the microscopist; the mechanism is intended to imitate the movements of the fingers as they shift the object about the stage. A

study of the figures \* will make my meaning elear.

A plate a (fig. 31) slides in and out under the fixed stage of the Mieroseope-stand. A U-shaped picce is cut out of its inner end, so that it in no way interferes with the condenser and substage apparatus. This wholc plate is worked by a doublo rack, one on each side of the plate, FIG. 31.



and the pinion 1. The plate is made to slide completely out, but for the mechanical (transverse) movement of slides it has a range of about 7 cm., which is, as far as I know, more than twice the range of any other meehanical table. When driven quite home, a projects about  $2\frac{1}{2}$  cm. from the side of the stage, so that it is not much in the way, and may be left in its place when the mechanical movement is not required.

The movement to and from the observer is obtained by means of the piece b which slides backwards and forwards aeross the end of a. It has a serew movement 2. In the example figured and described b has a range of about  $3\frac{1}{2}$  cm. But it is clear that this could be largely increased by widening the end of a. In the present case, in order to obtain greater range, a device is resorted to which will be described below.

Having thus got a mechanical movement of 7 by  $3\frac{1}{2}$  cm. at the sides

\* The engravings are from photographs kindly taken for me by Mr. C. J. Robinson, of 3, The Broadway, Streatham, S.W.

of our Microscope, the question is how to utilize it for the manipulation of slides. Here there is abundant scope for invention and adaptation to the different requirements of the microscopist.

The method here employed of attaching the slides to the piece b is by means of a bar, which we call the "arm" d. The arm passes through the head of a short stout pin c. This pin can be placed in one of three holes in b, according to the requirements of the objects to be studied. In order to prevent c from rotating in its socket a cross-bar passes through it, and lies in the groove seen in the figure to connect the holes in b. The arm d thus remains rigidly parallel with the plate a, and the movement of the pinions I and 2 will clearly carry anything attached to the proximal end of the arm freely across the stage of the Microscope in any direction. In order to give still further range, the arm is made to slide through the head of the pin, and the latter itself can be placed in any one of the three holes in b.

There are two methods of enabling the arm to move slides, each of which has its advantages.

If it is especially desired that the slide should lie flat upon the Microscope-stage, then it is perhaps best clipped by an arrangement



such as that shown in fig. 32, where the small arm K slides up and down, so as to take slides of almost any width ( $4\frac{1}{2}$  cm. in the one figured). The tip of K is provided with a piece of indiarubber tubing, which prevents slides from escaping when the clip is drawn from left to right.

Another plan is that shown in fig. 31, where the slides rest on a light frame *e*.

The clamp m slides backwards and forwards so as to be adaptable to any size of slide, trongh, &c.

These clips and frames are clearly the least expensive parts of the whole mechanism, and the microscopist can have several, of the sizes which experience teaches him are most useful for his special requirements.

It is obvious that instead of d carrying a frame or clip the end of the arm might be provided with forceps, and in this case the pin might be modified into a ball-and-socket arrangement for the moving of these forceps; we should then have the usual stage-forceps with, however, movements of much greater range.

The new stage as figured here is not made to rotate. There is, however, no mechanical difficulty in the way of making such stages to revolve. In order to attain this the piece n, with its corresponding piece at the other side of d, in which d slides, would have to be screwed to a revolving ring of the permanent Microscope stage. The revolution could not, however, be complete on account of the projecting part of a.

For the recording of the position of objects a scale is cut across the stage, and another along the edge of the frame or long arm of the elip, and the holes in b are numbered so that positions at the very opposito ends of even large culture plates could be noted without difficulty.

It remains to be stated that I have tested the stage with high and low powers, with large and small slides, with series of sections, with troughs, and with watch-glasses, and found that it fulfils all reasonable requirements. For watch-glasses a brass plate is either clipped or still better carried on a frame, the Microscope being placed upright. The watch-glass rests in a hole cut out of this plate. A plate of wood or of thick cardboard would of course serve almost as well as one made of brass.

The firm of Carl Zeiss is famous for the excellence of the work it sends out. If the new mechanical stage does not prove to be the boon to microscopists which I anticipate, the fault will lie in the design, and certainly not in the way the designs have been carried out. If, on the other hand, the stage or some modification of it be generally adopted, it will but add one more to the long list of valuable additions to the science of microscopy which have issued from the enterprising firm of Carl Zeiss of Jena.

### (4) Photomicrography.

**Photomicrography.**\*—Mr. A. Pringle gives a brief sketch of the various improvements in photomicrography, which have been made within the last two or three years. One of the most important is the improvement in colour-rendering, due to orthochromatic photography. Of no less importance is the remarkable improvement in optical methods which we owe to the so-called Jena glasses. In the production of these glasses it was found possible to obtain different relative proportions of refraction and dispersion, the result of which has been a set of objectives in which the achromatism reaches "an almost ideal point of perfection." The value of these apochromatics is more especially felt in photography, for hesides giving better correction these glasses also enable us to obtain a much greater angular aperture, and consequently more perfect definition.

Amongst slight improvements in the ordinary apparatus the author mentions the strong rigid support for the ocular end of the Microscope, which is a feature of the instrument designed by himself and Mr. Swift. This support is very necessary in work with high powers in order to prevent tremors during the exposures.

Having mentioned the more evident improvements in apparatus and methods, the author proceeds to treat of the various difficulties met with in high-class work. The first difficulty considered is that of illumination. For work with high powers it is essential that the substage condenser and the objective should bear some relation to each other in angular aperture and also in focal length. In low-power work the even

\* Journ. and Trans. Photographic Society, xvi. (1891) pp. 71–9. 1892. illumination of the object is best attained by placing, between source and object, the object-glass out of an ordinary low-power eye-piece. For the same purpose the author also invariably uses a bull's-evc.

With regard to illumination by monochromatic light, the author despairs of the method by means of a prism, owing to the uneven illumination of the field. The difficulties of rotundity and colour are next touched upon. The latter may generally be overcome by the help of orthochromatic plates and screens, except in cases where the staining is faint or faded.

In objects with very fine markings there is generally a tendency to a want of contrast in the result. Such difficulties are very hard to overcome, for, owing to the wide angular aperture required in these cases, the condenser cannot be dispensed with. With regard to increased magnification, the author's experience is that it is a mistake to try to strain a lens to more than ten times its initial power.

Focusing in Photomicrography.-M. P. Francotte communicates the following, which has been translated from his manuscript :--- "In photographing a microscopical preparation, two slides, one of ground glass and another of colourless polished glass, are used in order to focus the By the first of these slides only a rough focusing can be image. effected, but it is possible by its aid to appreciate the amount of light on the field of the Microscope and also to judge if the image is equally illuminated in all its parts. The second slide through the intervention of a lens allows of the exact focusing. But here the fact that the image is not visible to the naked eye is a source of great inconvenience; for it is impossible to bring back the object to the centre of the plate if it has been displaced during the operations. The lens which has to be moved about at a certain distance from the image formed on the glass is the only guide.

In place of this slide I have long made use of one of yellow, red, or slightly smoked glass (the last for photographing with sunlight). On a yellow glass the image can be seen by the naked eye, and all its details can be perfectly distinguished without the aid of a lens. Thus an exact focusing can be effected without losing the advantages afforded by the ground glass; for with the naked eye any displacement of the image can be observed, and by means of the lens the exact focusing can be effected just as with the colourless glass. Such are the advantages as regards the focusing of the image. But the tinted glass is of great service when the electric light or sunlight is used. The difficulty in focusing in the latter case is well known; the eyes are dazzled by the excessive light and it is sometimes necessary to forego the advantages presented by the polished glass and lens. This inconvenience is considerably modified by the coloured glass, and the focusing can be effected without trouble (with or without the lens) if the glass is tinted in proportion to the amount of light which falls on the plate. In our opinion all microscopical cameras should possess three slides, one of ground glass, another of glass tinted yellow or red or slightly smoked (like the glasses for moderating the light in the Abbe camera lucida). and lastly a third of colourless glass to be employed in special cases. With the tinted glass the results will be soon found to be far superior and the images much more detailed than before its use."

270

Van Heurck's Vertical Camera for Photomicrography. — This camera (fig. 33) was exhibited at the February meeting by W. Watson &



Sons and is constructed on similar lines to the one employed by Dr. Van Heurck for all his high-power work. It consists of a mahogany body U 2

# 272 SUMMARY OF CURRENT RESEARCHES RELATING TO

supported by four legs, with a focusing screen at the top, and at the lower end, a leather bellows, by means of which connection is made between the camera and the Microscope. On one side of the camera body, a large hinged door having rabbeted fittings is placed, and on opening this the head of the worker can enter the camera, and the eye be placed to the tube of the instrument to make the adjustments; the door is then closed and final focusing is done on the screen at the top of the camera. Messrs. Watson state that the advantages are, that all the motions of the Microscope are under the direct control of the hand, and that it can be at once placed over the Microscope, and a photograph taken, without the re-adjustments necessitated by the horizontal form of camera.

#### (5) Microscopical Optics and Manipulation.

Microscope Tube-length and Resolving Power.\* — Mr. H. G. Jameson writes: — "In the discussion of the relative merits of the English and Continental tube-lengths, one argument in favour of the English tube seems to have escaped notice, namely, that it gives, with any individual lens, a distinct advantage in resolving power. Taking the values for the different tube-lengths recently adopted by Prof. Abbe, the gain in resolving power for the English tube with a (true) 4 in. object-glass, is as much as 43 per cent., with a 2 in., 13 per cent., and with a 1 in. 5 per cent. With a 1/4 in. the difference falls to 1 per cent., and with higher powers it becomes imperceptible, but still a gain in definition of 5 per cent. with a 1 in. o.g. is not to be despised.

The advantage of the long tube may easily be observed practically by taking an ordinary optician's 4 in. objective (probably really about a 3 in.), and inserting a diaphragm behind the lens about 1/4 in. diameter, and examining the 1/100 mm. lines on a stage micrometer—it will be found that these are distinctly separable with a 10 in. tube, but vanish when it is shortened to the Continental standard.

This seems at first sight contradictory to Prof. Abbe's formula, which makes the number of lines per inch resolvable by any lens  $=\frac{2}{\lambda}$  N.A. But we have only to examine carefully the definition of numerical aperture, viz. N.A. =  $n \sin u$ , to see that N.A. is itself a variable quantity. The matter may be simplified by leaving out the term n, that is, by considering only non-immersion lenses, in which case N.A. =  $\sin u$ . Let R = the semi-diameter of the lens, f =its focal length, and T = the optical tube-length. Then, so long as the lens is focused at its true focal length, and the rays issue parallel beyond it,  $\sin u = \frac{R}{f}$ . But in practice, of course, the lens is withdrawn farther from the object, so that the rays converge above to form an image at a distance depending on the tube-length. Call this anterior conjugate focus f'. Then  $f' = \frac{Tf}{T-f}$ . And, therefore, in the practical use of the microscope, we get the modified expression, depending upon the value of

\* Engl. Mech., liv. (1892) p. 489.

T, N.A. =  $\frac{R(T-f)}{Tf}$ . It evidently follows from this, that measurement with the apertometer only gives the N.A. for the particular tubelength used in the experiment, and that a lens should not be described as of such and such N.A. without mentioning the length of tube with which it is intended to be used.

Of course the deficiency of resolving power with the Continental tube-length might be made up for by either increasing the diameter or deepening the curves of the lens. But this involves more careful correction and increased expense. So that the argument may be put this way: the short tube, in order to give the same results in the way of resolution as the long, requires to be fitted with a more expensive lens."

**Optical Theory of the Microscope.** The Virtual Image.\*—Schlor D. Joaquin Ma. de Castellarnau in this little treatise deals with the optical theory of the virtual image, a subject which has been somewhat neglected in most books on the Microscope.

The object of an optical instrument is to modify advantageously the retinal image with respect to the limits of visibility. The first section of the book is accordingly devoted to the consideration of these limits. The function of the eye as an optical instrument, the question of accommodation, the magnification of the retinal image, and the influence of diffraction on its formation, are all fully discussed. The conclusions arrived at are that when the eye fails to perceive clearly the form and colour of an object it is for the following causes:—The want of sufficient illumination as regulated by the aperture of the pupil; the smallness of the retinal image; the alteration which the image suffers from the effect of the diffraction resulting from the pupil; the fact that the eye does not receive and utilize in the formation of the image all the diffracted rays resulting from the passage of the light through the small elements of the object.

In the two remaining sections the author shows the effect of the simple and compound Microscope in extending these limits of visibility. In these sections the subjects of the amplifying power and the numerical aperture are fully treated.

#### (6) Miscellaneous.

Exhibition of Microscopes at Antwerp in 1891.t—This exhibition, which was chiefly due to the initiative of Dr. H. Van Heurck, was opened in the Athénée Royal, in Antwerp, on August 9th, 1891. The greater part of the exhibits was contained in an immense room, along one side of which was arranged a series of old Microscopes, illustrating the history and gradnal development of the instrument. Next to this came the fine collections of Dr. H. Van Heurck, the Microscopes of Watson and Sons, Zeiss, Powell and Lealand, and Nachet, the special collection of Prof. Bolsins, and the bacteriologieal apparatus of Adnet. Down the middle of the room were arranged the Microscopes of Reichert, various phases in the construction of objectives of Zeiss, the

\* 'Teoría Optica del Microscopio. La Imagen Virtual,' Cronica Cientifica, Barcelona, 1891, 105 pp. (29 figs.).

† Ann. de Microgr., iv. (1891) pp. 22-30, 69-96, 120-59, 199-219.

large photomicrographic and projection apparatus of the same maker, bacteriological apparatus from Paris, microtomes of Jung, Microscopes of Leitz, and preparations of Tempère and Thum. Against the opposite wall of the room were boxes containing numerous photomicrograms by Dr. Van Heurek, Thévoz, Müller, &c., and lastly, opposite the Paris exhibit came the apparatus from the bacteriological laboratory of Wiesnegg. The two shorter sides of the room were occupied by two large cases containing Microscopes of Hartnack, apparatus of electrical illumination by Trouvé, bacteriological apparatus of Rud. Seibert, microtomes of Erbe, &c. Above these cases, painted in letters of gold, were the names of famous microscopists and celebrated makers.

In an adjoining room were coloured photomicrograms on glass by Lumière, many photomicrograms of various substances used for purposes of adulteration, and a special exhibition by Möller of type-plates of preparations of diatoms.

The number of exhibitors was comparatively small, but the best firms were well represented, and the instruments to be seen were some of the finest that have been made. Bacteriology was the weak spot in the exhibition. The laboratories of the Belgian, German, and English universities sent no contributions, probably in great measure owing to the clashing of the Antwerp Exhibition with the bacteriological exhibition at the Hygienic Congress in London.

The various exhibits may be divided into the following sections :---

(1) Historical exhibition of microscopy.

(2) Microscopes and their accessories.

(3) Instruments and apparatus of bacteriology.

(4) Micrographic preparations, photomicrograms; and lastly, works on microscopy.

(1) Historical exhibition of Micrography.—Above the cases containing this exhibition was placed a portrait of Zaccharias Janssen bearing an inscription which stated that the compound Microscope was discovered about 1600 by Hans and Zaccharias Janssen. The authentic instrument constructed by the Janssens about 1600 formed one of the most interesting of the objects exhibited. Next to this instrument were four authentic Microscopes of Leeuwenhoek.

Among the compound Microscopes anterior to the 18th century was one with four glasses, of the form of the instruments constructed by Divini. It was mounted on a tripod, and was formed of two tubes fitting into each other, but was not provided with a mirror. Dr. Van Heurck exhibited a Microscope by Marshall (London, 1704), which approached in its arrangements the Microscopes of to-day; like them, it has a rapid sliding motion and a slow motion effected by a screw. Next to this were two Microscopes of Scarlet and Culpepper, which made their appearance in England about 1738. In these instruments the body-tube slides in a sccond tube supported on three feet; the stage is fixed, and the illumination is by a mirror movable in all directions, mounted on a foot.

Several Microscopes of Cuff dating from the middle of the 18th century served to show the progress which had been made in Microscope construction. They were provided with numerous objectives fitting into each other, with micrometer-screw, diaphragm, free stage, mirror and lens for the illumination of opaque objects. A Microscope by Brander (about 1745) was even more perfect, for, besides an excellent slow motion by micrometer-screw, identical with that adopted about 1835 by the German and French opticians, it also carried a micrometer-screw in the eye-piece.

Amongst other Microscopes of the 18th century exhibited was one of Dellebarre, presented to the Académie des Sciences de Paris in 1778, an inclining Microscope of Navin, a very fine instrument in copper gilt, with a curious slow motion and a screw motion allowing the horizontal displacement of the body-tube; and, lastly, the Microscope which was presented to Buffon by his pupils in 1748. Of the instruments of the beginning of the present century, the Microscope of Selligue, the universal Microscope of Ch. Chevalier, and the achromatic Microscope of Amici were the most conspicuous in the perfection of their design and workmanship.

(2) Microscopes and their Accessories.—The number of opticians who exhibited instruments was altogether only eight, viz. Powell and Lealand, and Watson and Sons, representing England; Hartnack, Leitz, Seibert, and Zeiss, Germany; Reichert, Austria; and Nachet, France.

The firm of Ernest Leitz exhibited eight Microscopes, designed more particularly for medical and bacteriological researches. Their stand Ia. is similar to the one figured in this Journal, 1889, p. 439, except that it is provided with a rotating stage, which can be centered by a screw. The Abbe condenser is moved by rack and pinion, and the iris-diaphragm can be displaced laterally by means of a pinion acting on a horizontal rack. The oil-immersion objective 1/12 of this firm, with numerical aperture  $1\cdot 30$ , was highly commended by the jury of the exhibition for clearness and exceptional resolving power.

The firm of Nachet was well represented. Besides the large model (No. 1) described in this Journal, 1886, p. 837, they exhibited a very perfect instrument specially designed for bacteriological work. Several instruments for photomicrography were shown by this firm; amongst them a large inverted Microscope for very high magnifications. The whole apparatus is, to avoid tremors, supported upon a large vertical tube which forms the base. Amongst the specialities of this firm, the adapter, which renders the change of objectives in their instruments so simple and easy, is most worthy of notice.

Powell and Lealand only exhibited one instrument, their stand No. I., which needs no description here. Accompanying the Microscope were their three apochromatic objectives, the 1/4 in. dry, aperture 0.95, the 1/8 homogeneous immersion of aperture 1.40, and the 1/10 homogeneous immersion of aperture 1.50, as well as compensating eye-picces and apochromatic condensers.

The firm of Hartnack exhibited their stands IV., V., and VI. In these the Abbe condenser has the usual disposition, and is provided with a slow motion; the iris-diaphragm can be displaced horizontally by rack and pinion. The special models for bacteriological work, like those of Zeiss, Leitz, Reichert, and Nachet, have a far from graceful appearance, owing to the wide separation of the body-tube from the vertical axis of the micrometer-screw. This mode of construction, which is rendered more unsightly by reason of the shortness of the Continental tube, is adopted, it is confessed, with the wholly unnecessary object of having a stage large enough to carry cultivation-plates.

M. Hartnack also exhibited a very remarkable photomicrographic apparatus, consisting of a Microscope in a horizontal position connected with the camera, which was on a stand supported by five levelling screws. The camera has some interesting peculiarities. The spindle which acts on the micrometer-screw fits into the centre of the support instead of being placed at the side, as in most instruments; the pinion which transmits the movement to the screw-head of the slow motion, does not act directly, but through the intervention of a system; the result of this is that the initial velocity given by the hand is considerably reduced, and accordingly the focusing is rendered more easy and precise.

The firm of W. and H. Seibert exhibited several of their instruments, readily distinguished by their characteristic appearance from those of other makers. In these Microscopes the screw-head of the slow motion is at the top of the vertical column which supports the instrument. In the stand No. I. there are three motions of the body-tube, the coarse movement by rack and pinion, the ordinary slow motion with the screwhead below, and a third extra slow motion, for use with very high magnifications, in front of the column which supports the body-tube. To this model can be fitted either a rotating stage or one provided with rectangular movements. Their stand No. II. is similar, but does not possess the extra slow motion. Stand No. III. is smaller, but, like the others, it cau be inclined and fixed at any augle, and is provided with a rotating stage, Abbe condenser, and iris-diaphragm.

W. Watson and Sons, amongst other instruments, exhibited the Microscope, designed by Dr. Van Heurek, which is described in this Journal, 1891, p. 399, and met with such severe criticism from Mr. Mayall.

The firm of Carl Zeiss was very fully represented. Most of the large models, as well as the large photomierographic and projection apparatus of this firm, have been described and figured in this Journal. One of the specialities of this firm is a sliding adapter. It eonsists essentially of a mortise slightly inclined in order to prevent displacement of the tenon ou which the objective is screwed. This tenon is formed of two pieces, the lower one of which carries the objective, and can be moved from back to front by means of a screw. A second screw, fitted to the sliding piece, gives a movement from right to left.

The firm of Carl Reichert exhibited several of their stands. The new large model Ia. is provided with a circular stage rotating on its axis after the English system. The movable stage, also for use with this model, admits of an exact displacement of about 25 mm. in two perpendicular directions. The stand II. is of simpler construction. In this model the illuminating apparatus is so arranged as to allow of the rapid change from illumination by the Abbe condenser to the ordinary illumination by the mirror.

The stand No. VII. of this firm is a useful instrument for students, or even for the laboratory. The stage is circular and very large; the micrometer-screw is sufficiently good to allow of the use of immersion objectives. Perhaps the most remarkable point about this instrument

276

is its price, which is only 65 francs. One of the specialities of this firm are the semi-apochromatic objectives.\*

The firm of Reichert also exhibited two forms of microtome. In one of these the object is moved in a vertical direction by a micrometerscrew, up to a limit which can be regulated at will. In the other form, the object is moved by micrometer-screw in an inclined plane.

(3) Instruments and Apparatus of Bacteriology.—The Municipal Observatory of Paris exhibited a great variety of apparatus for the analysis of air and waters. The so-called aeroscopic methods of Pouchet, Pasteur, and others occupied a large place in this exhibition. In the most recent registering aeroscope, the plate covered with lichen jelly on which the atmospheric dust is deposited, is provided with a clockwork arrangement which gives a circular movement to a glass disc on which 24 divisions, corresponding to the 24 hours of the day, are engraved.

The firm of Adnet exhibited various sterilizers and stoves of Sorel, Schribaux, Miquel, and others. Other exhibitors in this section were Wiesnegg, Seibert, and Trouvé.

(4) Micrographic preparations, Photomicrograms, Works of Microscopy.—In this section an interesting exhibit consisted of 550 preparations used by Dr. Van Heurek in the publication of his important work on the diatoms of Belgium.

Prof. Bolsius exhibited his new stage which allows of a lateral movement of 72 mm., and 10,000 sections which served in his researches on the segmental organs of the Hirudinea.

The exhibit of Mr. J. Deby included a large International Microscope of Messrs. Beck, with various accessories, a mineralogical Microscope of Seibert and Kraft, 50 slides representing the complete anatomy, by longitudinal and transversal sections, of *Brugmansia Lôwoi*, 30 sections of *Hydnophyton formicarium* and *Myrmecodia tuberosa* from Japan, &c.

Other exhibitors in this section were Tempère, Keller, Thum, and Möller.

Photomicrograms were exhibited by Dr. Van Heurck, Jules Van den Berghe, Otto Muller, M. Gife, Andrew Pringle, and others.

Simple Method of Drawing Microscopical Preparations.<sup>†</sup>—Mr. A. Hopewell Smith writes :—" There has always been a certain amount of difficulty attending the use of the camera lucida or Beale's neutral tint reflector for the above purpose. The twisting of the head into an uncomfortable position, the great fatigue to the eyes, and the by no means easy task of viewing both image and pencil at the same time, add to the troubles of making a faithful likeness of the object on paper.

To those especially who do not possess a camera lucida, or Beale's instrument, and to microscopists generally, I recommend the following arrangement of ordinary apparatus:—The Microscope body is placed in a horizontal position, and the mirror removed from its substage attachment. The Microscope slide having been placed on the stage, the illuminant (lamplight for choice), is condensed on the slide by means of a bull's-cye in the same way as for photomicrography. Care must be taken to centre the light. The concave mirror is thon

\* See this Journal, 1890, p. 93.

† Journ. Brit, Dental Assoc., xiii. (1892) pp, 78-9.

attached to the front of the cye-piece of the Microscope by a piece of thin wood or a spring, and has its surface at an angle of about  $45^{\circ}$  with the plane of the anterior glass of the ocular. The image is thus projected on to the paper beneath. No distortion will occur if the outer ring of light is *perfectly* circular. A dark cloth, such as photographers use, is thrown over the draughtsman's head, and also the body of the Microscope, and all light excluded save that through the Microscope lenses. Any section can thus be easily, rapidly, and comfortably drawn, and accurate representations of objects magnified up to 500-600 diameters can be obtained."

Walter H. Bulloch.-Mr. H. L. Tolman, President of the Illinois State Microseopical Society, communicates the following notice:-"The death of Walter H. Bulloch, of Chicago, the eminent Microseope-maker, is a severe loss, not only to our Society, of which he was for nearly twenty years a prominent member, but to the eause of science at large, and a short sketch of his life and work will not be uninteresting. Mr. Bulloch was born in 1835, at Glasgow, Scotland, and lived there until he was seventeen years of age. About 1852, the family emigrated to New York, where Walter learned the trade of tailor with his father. But his innate fondness for mechanical pursuits made him dissatisfied with his prospects, and he was apprenticed to Messrs. Pike and Sons, then a leading firm of opticians and instrument-makers, on Broadway, New York City. After serving his time, he went into business on his own account, until the war of the rebellion broke out, when he enlisted as a private in the 12th N.Y. His term of service, however, was very short, as he con-Volunteers. tracted a severe cold, which developed into rheumatism, incapacitating him from further work, so that he was mustered out of the service. Returning to New York, he formed a partnership with William Walcs, the well-known maker of objectives, and continued in business there until 1866, when he moved to Chicago. He was very successful, and had accumulated considerable means, when his shop and tools were destroyed in the memorable Chicago fire of October 8-9th, 1871, and Mr. Bulloeh sustained a financial loss from which he never recovered. Immediately after this misfortune hc went to Boston, and was for a time connected with the late R. B. Tolles, but soon returned to Chieago. In 1888, he accepted a position in the Bureau of Weights and Measures, under the Government, but he chafed under the restraints of an official situation. and after six months' experience returned to his home here. Before he left his health had begun to fail, and after his return late in the fall of 1890, he suffered still more. But his indomitable perseverance led him to struggle on. He opened a place at 303, Dearborn Street, in a very advantageous business portion of the city, and began work again. It was not for long. After struggling with disease for about six months. he was compelled to stop work for ever. He died Nov. 5th, 1891.

Mr. Bulloch was a man of pronounced character and indomitable energy and perseverance. To those who did not know him well, he appeared brusque and sometimes even overbearing, but his numerous friends soon learned to appreciate his straightforward manner of expressing his views, his pertinacious but just demands for a proper recognition of his rights, and his outspoken criticism of what he deemed erroneous in the theories or opinions of others. In his business he was conscientious and painstaking to a fault. Often when making an instrument or piece of apparatus to order, if he saw where there was room for improvement, he would spend hours or days in experiments, perhaps wasting all the results of his previous labour, refusing to slight his work at any cost. Whether it was the simplest accessory or the finest Microscope stand, nothing was allowed to leave his work-bench until it was as perfect as his trained hand and eye could make it. His reputation was more than money, and he lived to see his fame world-wide. Besides being a member of the Illinois State Microscopical Society, he was a member of the Chicago Academy of Sciences, the American Society of Microscopists, and of the Royal Microscopical Society of London. His death leaves a gap in the rank of scientific workers which cannot easily be filled."

#### β. Technique.\*

### (1) Collecting Objects, including Culture Processes.

Simple Apparatus for Cultivation of Small Organisms. +- Prof. Marshall Ward describes a simple apparatus for the cultivation of small organisms in hanging drops and in various gases under the Microscope. The two ends of a piece of thick-walled glass tubing about 3/4 in. in diameter and 3 in. long, are softened and slightly drawn to narrow tubes, not too thin. One face of the now central bulb is ground flat until a hole about 1/2 in. in diameter is cut through; a similar hole is then ground on the opposito face of the bulb. The glass is sterilized at 150° C. and cemented by paraffin (or by gelatin in acetic acid) by one of its ground faces to a broad glass slide properly sterilized. Sterilized cotton wool is stuffed into the two narrow tubulures, and the hanging-drop culture, properly prepared on a sterile cover-slip, is cemented over the upper hole of the chamber. If it is necessary to pass gases into the culture one of the stuffed 'tubulures is connected by means of caoutchouc tubing (sterilized in corrosive sublimate, absolute alcohol, and boiling) with the appropriate gas apparatus. If a very strong coverslip and careful cementing are employed a very good partial vacuum can be obtained and even retained for some hours. The apparatus seems to be adapted for many kinds of examination.

Macaroni as a solid Nutrient Medium.<sup>‡</sup>—Prof. G. de Lagerheim recommends macaroni as a substitute for potato. Macaroni as white as possible, and having a diameter of 5 mm. and a lumen of 3 mm., is broken up into pieces of 4.5 cm. and placed in a sterilized test-tube, and then water sufficient to cover the macaroni by 1 cm. is poured in. The tube is then heated for about a quarter of an hour, by which time the macaroni is soft and swollen; the water is then carefully poured off and the testtube, having been plugged with cotton wool, is steam-sterilized. Thus

<sup>\*</sup> This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes;
(4) Staining and Injecting; (5) Mounting, including slides, preservative fluids, &c.;
(6) Miscellaneous.
† Rep. Brit. Ass., 1891 (1892) pp. 678-9.

<sup>‡</sup> Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 147-8.

prepared the macaroni is almost quite white, and hence for chromogenic bacteria is an effective medium as the coloured colonies show up well against the white backing.

Preparing Ammoniated Gelatin, and cultivating Bog-water Bacilli.\*—For cultivating various Schizomycetes, Herr F. Pohl recommends gelatin which has been alkalinized by means of carbonate of ammonia, and this medium seems specially adapted for putrefactive organisms and for the long series of spirilla, for the development of which an alkaline medium and much oxygen are necessary.

Gelatin cannot be alkalinized with carbonate of ammonia directly, and sterilized in the usual way, for then it very soon liquefies and loses its capacity for setting. The gelatin and carbonate of ammonia must be sterilized previous to being mixed, although for safety the mixture may be heated in a water-bath for half an hour, but not longer, otherwise the ammonia will have volatilized and the gelatin will not set.

The gelatin was made up with bog-water, and the medium used for cultivating bog-water bacilli, of which four new species are described by the author, *B. stoloniferus*, *B. incanus*, *B. inunctus*, and *B. flavescens*. Besides gelatin, agar and potato were used as cultivating media, and their behaviour towards starch, milk, and sugar was also examined. None wcre pathogenic; the first two turned sugar into alcohol, but diastase, indol, and phenol were not detected; the last two possessed strong liquefying action, peptonized milk without coagulating it, and converted sugar into alcohol.

Epidemics among Mice kept for experimental purposes.<sup>†</sup>—Prof. L. Loeffler narrates the history of two epidemics among the white mice kept for experimental purposes at the Hygienic Institute at Greifswald.

In the first he was able to identify the micro-organism found in the bodies of the mice with the microbe of mouse septicæmia, an interesting discovery, because the infection had in all probability attacked the animals through the digestive tract, while it was first described by Koch as being intimately associated with experimental (artificial) traumatism. The total loss of life in this epidemic was fifteen.

In the second epidemic 31 out of 45 animals died in the course of four weeks. The dead animals were found partially eaten; it was therefore probable that the disease had been handed on. The postmortem appearances were variable, the most prominent being a swollen brownish-red firm spleen, inflammation of the intestinal mucosa and swelling of the mesenteric lymphatic glands, a collection which at once recalls enteric fever. From all the dead mice was isolated a short bacillus, showing lively movements when observed on the hollow slide, and having numerous flagella when stained by means of an alkalinized mordant.

The bacilli were cultivated on the usual media—gelatin, agar, bloodsernm-pepton-sugar-bouillon, potato and milk. Neutral fluid media were rendered acid, gas bubbles were disengaged, and by the iodoform reaction alcohol was demonstrable in the distillate. Sections of the discased organs showed that the bacilli were chiefly massed in the

<sup>\*</sup> Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 141-6.

<sup>†</sup> Tom. cit., pp. 129-41.

eapillaries, and formed aggregations similar to those found in enteric fever, whence the author calls this bacillus *B. typhi murium*.

From experiments made with this bacillus the author found the duration of the disease to be thirteen days, and that the virus was introduced into the system through the digestive tract, and concludes that the great sensitiveness of certain kinds of mice to this microbe suggests the possibility that it may be of great practical value in husbandry. The experiments further showed that the short-tailed field mouse (Arvicola arvalis) was extremely sensitive to the presence of B. typhi murium, while the long-tailed field mouse (Mus agrarius), cats, rats, dogs, small singing birds, pigeons, guinea-pigs, and rabbits were quite unaffected by it.

The author concludes by speculating on the origin of the disease, negativing the hypothesis that the *B. typhi murium* might be a variety of some of the pathogenic bacteria studied from time to time in the Greifswald Institute, and that it was due to something in the food; the latter supposition is disproved by the fact that only mice in a particular cage caught the disease. Altogether three epidemics of this disease have occurred.

Fodor's Bacteria-fisher.<sup>\*</sup>—Prof. J. Fodor has devised an instrument which he calls a bacteria-fisher, for removing from a cultivation plate a specimen of any particular colony, with certainty and without contamination. The apparatus, no illustration of which is given, is mounted on a Microscope stand, and consists of a series of rods and rings, fixed at various angles, and maintained in position by screws. At the end of the apparatus is a glass rod carrying a platinum needle. By altering the length of the various rods, and fixing them at different inclinations, the needle is brought under the Microscope, and in contact with the colony from which it is desired to remove a specimen. By this means tolerable certainty is arrived at, as the trembling of the hand is avoided, and the position of the needle can be seen.

Bacteriological Examination of Water.<sup>†</sup>—Dr. F. Kräl, in order to obviate the great inconvenience arising from the size of some of the apparatus employed in the bacteriological examination of water, has devised an apparatus consisting of so many flat glass capsules fitting in a case. Each capsule has a diameter of about 9 cm., the sides are 4 mm. high, and the glass 1 mm. thick. The top and bottom of these capsules are quite flat, and can therefore be placed directly under the Microscope. Twenty of such covered capsules are, when piled one on top of the other, about 15 cm. high.

In order to hasten the setting of the gelatin, or to prevent any subsequent liquefaction, a double tin case may be used, so that the jacket can be packed with ice.

Keeping Cestoda alive.<sup>‡</sup>—Dr. E. Lönnberg records some fourteen experiments made for the purpose of keeping Cestoda alive under artificial conditions. *Triænophorus nodulosus*, a parasite of pike, was selected because it is casy to obtain. The basis of the medium was a slightly

<sup>\*</sup> Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) pp. 721-2.

acid pepsin-pepton solution, containing from 3 to 4 per cent. of nutriment, and less than 1 per cent. of NaCl. In most experiments some slight modification was adopted, e.g. more water, olive oil, grape sugar, &c., were added. In the least unsatisfactory experiment the worms lived from the 26th of one month to the 28th of the next.

The great difficulty in the way of a signal success seems to have been the occurrence of putrefaction in the medium, which had frequently to be changed.

The author thinks that the composition of the medium has less to do with the unfavourable results than the decomposition, since the particular medium in which the worms survived for more than four weeks little resembled the conditions natural to the animal.

It is considered that these experiments favoured the notion that besides requiring a suitable temperature and certain mechanical conditions, the development of tape-worms within the host is aided or prevented by the reaction of the intestinal contents, e.g. too great acidity being inhibitive.

Collection and Preservation of Diatoms.\* — M. J. Tempère gives instructions in the best mode of collecting and isolating diatoms, and preparing them for examination. A convenient form of pocket-drag is described, made of metal, which can be used for rocky bottoms or places encumbered with solid bodies. A list is appended of all the more important beds of fossil diatoms.

#### (2) Preparing Objects.

Study of Structure of Protoplasm.<sup>†</sup>—The editor (Dr. A. C. Stokes) writes :--- "According to Heitzmann, Klein, and several German observers, animal protoplasm is a network of fibrils radiating in all directions through the cell, and containing a homogeneous fluid within its meshes. This and other inclosurcs have been referred to in an admirable manner by Prof. Kirsch in his valuable series on cytology; but a sight of this reticulation within the cell is one that has been most desirable for every microscopist, but that to the amateur has been especially difficult. The appearance of the structure has been repeatedly figured, but the sight of a picture is not to be compared in satisfaction to the sight, although it may be an imperfect one, of the object itself. This has heretofore not been an easy task, but through no discovery of my own it has recently come to my knowledge that there is a common animal in whose intestinal cells this structure of the protoplasm may be observed with comparative ease and with lively satisfaction. The animal is . . . Oniscus murarius. In order to see the appearances as described by certain observers 1 sought the Oniscus, and in five minutes had gathered a dozen. . . . Kill an Oniscus by a few drops of alcohol. Remove the legs, to get them out of the way. With fine-bladed seissors slit up the body on the lower or abdominal side. Push away, or carefully remove, the walls of that part of the body, and the intestine will be in plain sight. It is a nearly straight, tubular vessel, large for the size of the animal, and usually gorged with food or its remains. It is

\* Le Diatomiste, i. (1891) pp. 41-2, 46-7, 61-4 (3 figs.).

† Microscope, xi. (1891) pp. 276-8.

a conspicuous object, and may be readily removed. After removal it must be slit open, so that the inner surface shall lie upward toward the objective. To do this, my plan is with great care to insert a fine needle into the lumen, the cavity of the intestine, and when the tube has been placed on the steel without having its wall pierced at any point, it is gently rubbed with another needle until it is slit from end to end. This is not a difficult thing to do if the needle be fine and the microscopist's hand be steady. After the intestine, or a part of it, has been taken from the body, all subsequent manipulations may and should be performed on the slide and in the cell in which it is to be mounted.

The intestine, slit open, is then placed in a small drop of water, and while it still remains attached to the needle is to be gently freed by another needle and floated in the water, inside upward. The intestinal contents are then to be washed away by the repeated dropping of water. When thoroughly cleaned, as may be known by the disappearance of all the brownish freees, drain off the water and add one or two drops of a solution of methyl-green, one of the anilin stains to be had of the dealers in microscopical supplies. The dye aets rapidly, so that from four to five minutes will usually be long enough to colour the parts sufficiently. Wash away the superfluous stain, drain off the water, add a drop of diluted glycerin and apply the cover-glass, cementing it in place with shellac.

A small piece of the intestine is all that is needed; it is not necessary to take the entire intestinal tube. I have found the rectum, the posterior region nearest the external aperture, to be free from fæcal matter, and beautifully transparent. In this part, too, the cells may be rather better displayed than in anterior regions. The cells are comparatively immense, with conspicuous cell-walls, prominent nuclei with their inclosed nucleoli, and with the structure of the protoplasm finely displayed. The latter may be seen well with a good 1/5-in. objective, but of course with greater satisfaction under a lens of higher power.

A close, small meshed network of the protoplasm does not seem to be a constant and invariable feature of its structure, although in many cells it is exquisitely demonstrable, while in many others it is composed of exceedingly fine fibres radiating in every direction from the centre toward the cell-wall and forming meshes so narrow that they are very inconspicuous, really demanding somewhat careful search to see them. Here the fibrillated structure dominates, and this appearance calls to mind the aspect of an almanac sun, the face of the symbol being here represented by a somewhat irregular nucleus, with fine rays increased to an indefinite number.

While examining these large and beautiful cells the observer should bear in mind these two appearances, and not be disturbed if the network is not as plainly visible and the meshes as close, small and regular as he expected they would be. In my experience the fibrils are the most readily demonstrated, unless a very high power, a 1/12 or higher, be used to study the protoplasm surrounding the tubule of the nucleus and situated between it and the membrane including the nucleus and separating it from the protoplasmic contents of the cell. In this part of the object the reticulum, or network of delicate fibres, is superbly demonstrable. 284

Those that have been reading Prof. Kirsch's paper on cytology have learned that the nucleus, while it appears to be formed of a reticulation or network, is in reality composed of a single fine tubule much convoluted upon itself, the apparent network being produced by the crossing of the tubule over its preceding convolutions. The nucleus is in structure only a single, very much twisted tube, whose hollow is filled with a substance that has been named the nuclein. In the intestinal cells of *Oniscus* beautiful optical sections of this are obtainable, since the nuclein takes the stain with great avidity. The network is here conspicuous, and the meshes, unlike those of the protoplasm of the cell, are fine and small. Altogether, therefore, the intestinal cells of this common animal cannot be excelled as objects in which to examine the structure of the protoplasm—a subject that is always interesting, and should be seen and understood by every microscopist."

Methods of Technique in Embryology of Frogs.\*-Mr. T. H. Morgan describes his methods as follows :- " The eggs, during the periods in which it is difficult or impossible to remove the inner jelly membrane, can be freed in the following manner. With a pair of sharp scissors each eggmust be cut out from the general jelly-mass, retaining as small an amount of surrounding jelly as possible. It is then put into an alcoholic solution of picric acid for an hour or longer (one to twelve). The solution is prepared by saturating 35 per cent. alcohol with pieric acid, and adding the same amount of sulphuric as in Kleinenberg's solution. The solution is not diluted, but used saturated with picric acid. The eggs are then washed for several hours in 35 per cent. alcohol, several hours in 50 per cent, alcohol, and placed in 70 per cent. for several days, changing the alcohol once or twice if necessary. About the second day the inner membrane begins to swell, due to a slow osmotic action, I think, as the membrane is stretched by tension from within. On the third or fourth day the swollen membrane may be pierced by a sharp necdle, and the egg taken out, which is then placed permanently in 80 per cent. alcohol. The method is exceedingly simple, and consists largely in waiting a few days for the osmotic action to take place. Such eggs, if properly prepared, are in excellent histological condition. This simple method has proved so successful that I have not further experimented with it. It is possible that it may be improved by varying the strength of alcohol used, but I have not seen the need of looking further. The membrane does not swell in stronger alcohol than 70 per cent., and weaker would maccrate the eggs.

Certain precautions are necessary in imbedding the eggs to prevent brittleness. This is obviated by soaking the eggs before imbedding, for several hours, in a solution of turpentine saturated with paraffin, and kept in a warm place—not so hot as the water-bath ( $50^{\circ}$  C.). Heat causes the egg to become brittle. This is obviated by the above process of soaking, so that the egg need not remain so long as an hour in the melted paraffin of the water-bath.

In the younger stages there is no need for very thin sections, but sections 10  $\mu$  thick are sufficient for all purposes. If the sections are cut too thin the yolk tends to break up and crumble."

\* Amer. Nat., xxv. (1891) pp. 759-60.

Observation of the Process of Fecundation.\* - M. L. Guignard recommends the following method of preparation for observing the various stages in the process of fecundation, whether of animals or plants, especially the part played by the "directing spheres." Tho best fixing material is absolute alcohol, either pure or with the addition of from 0.2 to 0.3 per cent. of corrosive sublimate or picric acid. A 1 per cent. aqueous solution of sublimate, a saturated solution of picric acid, and a 0.5 per cent. solution of chromic acid, also give good results. In treating the cells themselves with vapour of osmic acid, it is necessary to note the exact time necessary and sufficient for complete fixation, too long exposure injures the staining. The preparation may then be hardened, first with Flemming's fluid, then with absolute alcohol. Flemming's fluid does not answer so well with tissues which are to be fixed en masse, such as pollen-cells or ovules, as with the contents of the embryo-sac extracted from the ovule after fecundation. The best staining reagent for the "directing spheres" is hæmatoxylin.

Study of Spermatogenesis.<sup>†</sup>—Dr. C. Pictet recommends three modes of procedure in the investigation of the delicate seminal cells-examination of living cells, various modes of dissociation and examination in more or less indifferent fluids, and sections. The last, however, is of little use. Examination of the living cell is most important, as there is no known reagent that does not alter it. The nucleus has generally been studied in a solution (1 per cent. to 3 per ceut.) of acetic acid iu water; dahlia (St. George's formula) or methyl-green (Carnoy) have been almost exclusively used as staining reagents. Good preparations havo been obtained by the vapour of osmic acid, followed by pyrogallic acid; and by chloride of platinum and permanganate of potash. must be remembered that the accessory nucleus is destroyed by acetic acid; when that body is to be retained, it is advisable to use a watery solution (5 per cent. to 10 per cent.) of chloride of manganese, to which a few drops of a concentrated aqueous solution of dahlia havo been added; this reagent is strongly recommended by the author.

Study of Development of Oviduct of Frog. 1-Mr. E. W. MacBride propared his tadpoles either with corrosive sublimate or with Perenyi's fluid and alcohol. Decalcification was effected by nitrie acid in strengths varying from 1 to 10 per cent.; strange to say, no difference was obsorved with different strengths, but a 3 per cent. solution for twentyfour hours may be recommended. Material preserved in picric acid was found to be quite unsuitable. The method of study was solely that of a series of transverse sections, but it is well to first remove the greater part of the gut, almost all the liver, the heart, and most of the lungs.

Study of Neomenians.§-M. G. Pruvot found that the best fixing reagent was corrosive sublimate concentrated at freezing-point; coloration en masse was very well effected in three or four hours by alumcarmine. Double staining, after sectionizing, by a watery solution of hæmatoxylin and eosin gave very fine preparations; it has the advantage

- \* Ann. Sci. Nat. (Bot.), xiv. (1891) pp. 166-9.
  - Mittheil. Zool. Stat. Neapel, x. (1891) p. 75.
     Quart. Journ. Micr. Sci., xxxiii. (1892) pp. 273-4.

  - § Arch. Zool. Expér. et Gén., ix. (1891) pp. 701-2.

1892.

of not destroying, as does alum-carmine, calcareous spicules, and certain elements, such as those of mucous glands, are much better stained by it; these glands are, however, preferably stained with methyl-green, which has a remarkable power of selecting mucus. After staining, the sections should be placed for a few seconds in a very weak alcoholic solution of methyl-green.

Preparation of Gastrulæ of Aurelia flavidula.\*—The embryos examined by Mr. F. Smith had been killed with picro-nitric acid and preserved in 90 per cent. alcohol for three years. Ehrlich's acid hæmatoxylin was found to be the best staining reagent for sections. Czokor's alum-cochincal stains embryos of different ages with corresponding degrees of intensity, increasing with the age of the embryo.

Examination of Spongicola fistularis.<sup>†</sup>—Mr. W. R. Melly found it very difficult to kill this curious medusoid in an extended condition. The best specimens for sections were those treated for two minutes in 1 per cent. osmic, and then passed through various stages of alcohol up to 90 per cent., hardened in absolute, and imbedded in paraffin. It was found best to leave the *Spongicola* in the Sponge, and dissect it out after hardening in absolute alcohol.

A Medium for preserving the Colours of Fish and other Animals.<sup>‡</sup> --Mr. A. Haly, Curator of the Colombo Museum, has for some years been making experiments to discover a medium which will preserve the colours of fish and other animals. The following is taken from the last Annual Report of the Colombo Museum:-- "In my last year's report I made some remarks on the use of carbolized oil as a mounting fluid for specimens already prepared by other means, the idea that it was a preservative in itself not having occurred to me. Further experiments this year seem to show (I do not like to speak too confidently in a climate like this, even with twelve months' experience) that it is one of the most perfect preservatives known both for form and colour.

Coco-nut oil and carbolic acid freely mix in all proportions. The mixtures at present under trial are oil raised to the specific gravity of 10° and 20° below proof-spirit by the addition of acid. Whilst the gum and glycerin process is absolutely useless for any animals, except certain families of fish, this mixture is good for every kind of vertebrate. The most delicate frogs are quite uninjured by it, and snakes undergo no change. The delicate plum-like bloom on the geckoes, the fugitive reddish tint on such snakes as *Ablabes humberti*, are beautifully preserved by it.

Another most important use is in the preservation of large fish-skins, which can be packed away in it for an indefinite period, aud mounted when wanted. These skins do not require varnishing, neither do they turn brown, but although, of course, they do not preserve their sheen like fish in the oil itself, they always maintain a silvery and natural appearance, quite different from that of ordinary museum specimens. If ever we get a new fish gallery, a show of our large species prepared in this way would form a most effective exhibition.

- \* Bull. Mus. C. Z., xxii. (1891) pp. 115-6.
- † Rep. Brit. Ass., 1891 (1892) pp. 367-8.
- \* Nature, xlv. (1891) p. 212.

It appears also to be a most excellent preservative for Crustacea and the higher orders of Arachnida; and also for centipedes, but it has hitherto proved a failure for marine invertebrates in general. It must be remembered, however, that the perfect miscibility of the two liquids opens up endless possibilities. Its absolutely unevaporable nature makes it invaluable in a tropical climate, quite apart from its other qualities.

With regard to this last remark I take the opportunity of stating that the acid enables coco-nut oil and turpentine to be mixed together. This forms a splendid microscopic fluid, in which objects may be allowed to soak without any previous preparation, and in which they become very transparent. A minute species of Crustacean, of the order Copepoda, and the leg of a fly, simply laid on a slide in a drop of this fluid and covered with an ordinary covering-glass, without any cell being made or cement employed, have lain on my table unaltered for the last ten months, and I cannot help thinking that such a medium as this cannot fail to prove a great boou to all workers with the Microscope."

Method of making Leaves transparent.\*-Dr. A. C. Stokes writes, "It frequently happens that the amateur microscopist would study the epidermal cells and appendages of the almost infinite variety of leaves, the structure of the cellular parenchyma, or body-substance of the leaf, the peculiarities of the cells and vessels of petals and of other parts of flowers. That is, he would if he could. It is sometimes an easy task to strip off the epidermis and to examine its cells, while in other cases it is almost impossible. Many chemical mixtures have been recommended for the purpose, and they accomplish the object after a fashion. The structure of the body of the leaf may be satisfactorily studied in sections, but not every microscopist can have a good microtomea poor one is an abomination. There is also much to be learned and much beauty to be seen in the petals of flowers and in the cuticle and cells of the anthers, but it has been almost impossible to succeed here without special and somewhat complicated processes. Yct there is a way to make these objects either entirely transparent or sufficiently translucent to render their study pleasing and comparatively The dealers will supply the microscopist with mounts of entire easy. flowers, made beautifully transparent, but the method of accomplishing this is not detailed with any spontaneity; indeed the preparers, so far as I have been able to observe, are deaf and dumb when the subject is mentioned in their presence. I possess a fine slide of tho entire flower of the common Houstonia, or "innocence," perfectly transparent, so that the cells of the epidermis, of the substance of the petals and of other parts, and the anthers with the pollen-grains in situ, may all be examined with a high power. How the thing was accomplished I have, until recently, been unable to ascertain. The secret has been so well kept that, so far as I can learn, only the dealers knew it; the books have not discovered it. Yet by a very simple method these objects, as well as leaves, may be made entirely or almost transparent, so that the vessels and the cells may be studied at one's leisure and in comfort.

\* Microscope, xi. (1891) pp. 265-7.

By this treatment the hair-like and glandular appendages and stomata are preserved in place and in structure, the protoplasmic contents alone being contracted toward the centre of the cells. It is a method that I have stumbled on by accident, but one that I can recommend to the microscopical botanist that desires to examine these parts without destroying or disarranging any of the constituents.

Place the petal, the anther, the whole blossom, or a part of a leaf on the slide, in a large drop of glycerin. See that it is completcly submerged beneath the liquid, and add a large cover-glass. It is best to use a slip without a cell. Then boil the glycerin over the lamp-flame until the parts arc entirely transparent or at least translucent, a condition that will arrive in a short time. Do not allow the boiling to be so violent as to disarrange the thin glass; let it be so gentle that the bubbles will run one by one to the edge of the cover and there break. If the glycerin should become discoloured, as will often happen when leaves are under treatment, draw off the liquid by a wet cloth and add fresh glycerin, repeating the process and the boiling until the leaf is saturated. The use of glycerin and the saturation of the cells form the secret of the process. The saturation is easily accomplished with petals and similar delicate parts; with thick and opaque leaves the time demanded is longer, and the specimen may become only translucent. I have made the thick and opaque leaf of the garden geranium, Pelargonium, so translucent that there was no difficulty in examining the hairs on the surface, the epidermal cells, the parenchyma and vessels, with the cells of the epidermis on the opposite surface. Of course there is a limit to the thickness and to the opacity that can be overcome, yet the method will be found exceedingly useful. Leaves and petals do not entirely lose their colour, although they become beautifully transparent. Of course the specimens must be permanently preserved in glycerin.

The secret that the dealers have seemed to keep so carefully, and that the books have ignored because apparently their authors had not learned the process, is here placed at the reader's disposal. I am sure that he will be pleased with the result of his experiment, and that he will find the objects so often mentioned, rendered easy of examination. Petals and other parts of the flower need no previous preparation. It is well, however, to cut the leaves so that there shall be two or more open surfaces for the penetration of the glycerin. In some very delicate specimens this will not be necessary; it is so when the leaf is thick or very opaque."

Preparing Agarics.\*—M. Fayod says that the best way to preserve specimens of agarics intended for microscopical examination is to let them dry slowly. The best way to do this is to place them in paper capsules and then to inclose them in cardboard boxes.

Such specimens when ready are to be cut dry. The sections are placed in water containing a little ammonia, if the filaments be thick-walled, and in strong ammonia or dilute potash if the wall be thin.

The specimens may also be preserved in spirit, and the spirit may

\* Ann. Sci. Nat., ix. (1889). Sce Rev. Gen. de Bot., iii. (1891) pp. 427-8.

288

### ZOOLOGY AND BOTANY, MICROSCOPY, ETC.

be economized by leaving the preparation only two or three days therein, and then, while still saturated, placing them in a paper capsulc, upon which may be written any necessary observations. Numbers of theso capsules can then be placed in wide-mouthed bottles plugged with cotton wool soaked in spirit, previous to being corked. Only slices from the middle of large species should be preserved, but the whole specimen should be previously hardened in 80 or 90 per cent. spirit.

### (3) Cutting, including Imbedding and Microtomes.

Method for Saturating Preparations with Paraffin.\*-Instead of dehydrating tissues or pieces intended for paraffin imbedding, the following method is recommended by Przewoski as being more economical, safer, and more easily applied than absolute alcohol :---After removal from ordinary spirit the preparation is immersed in anilin oil for 24 hours at least. It is then wiped, and the anilin oil removed by soaking in chloroform for 24 hours.. It is then immersed in parafin dissolved in chloroform (40 per cent.), and the next day in melted paraffin, which must be cooled down as soon as possible, to prevent it becoming brittle. The anilin oil may be previously dehydrated by distillation or by dropping a piece of caustic potash therein.

#### (4) Staining and Injecting.

Demonstrating the Plasmodium Malariæ.†-Herr E. Malachowski recommends the following method for staining the plasmodium malariæ. Hardening in alcohol, floating the cover-glass in a mixture of cosin solution and dilute aqueous borax methylen-blue solution. The red discs are grey or yellowish-red, the nuclei of white corpuscles red-violet, the plasma of the mononuclear leucocytes blue, that of the multinucleated pale violet, the plasmodia are blue, and certain granules within them, which have some relation to sporulation, red-violet.

Herr J. Mannaberg ‡ describes a new method for demonstrating the parasites of malaria. The preparation, dried in the air, is placed for 12-24 hours in a mixture of equal parts of saturated picric acid solution and distilled water to which 3-5 per cent. acetic acid has been added. It is then quite decolorized in spirit, and having been over-stained with alum-hæmatoxylin solution, differentiated with 25 per cent. hydrochloric acid alcohol and dilute ammonia alcohol. By this method the red discs and the plasma of the white corpuscles remain unstained, the hæmatoblasts are faintly coloured, while the nuclei of the leucocytes and tho chromatin of the plasmodium arc well stained.

The author describes the young plasmodium as consisting of a thin layer of non-pigmented plasma, a large round nucleus situated excentrically, and containing a nucleolus. In process of time the plasma differentiates into two layers, an ecto- and endoplasma; and ultimately the plasmodium is distinguishable into its plasmatic and nuclear halves.

<sup>\*</sup> Centralbl. f. Allgem. Pathol. u. Pathol. Anat., 1890, No. 26. See Bull. Soc.

Belge de Microscopie, xliii. (1891) pp. 12-13. † Centralbl. f. Klin. Med., 1891, pp. 601-3. See Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) p. 706.

<sup>1</sup> Tom. cit., p. 513. See Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) pp. 705-6.

290 SUMMARY OF CURRENT RESEARCHES RELATING TO

In the nuclear half small granules appear, "the nucleoli of the spores," which later on develope a nucleus and plasma. The plasmatic half seems sometimes to participate in this process of subdivision, and sometimes to take no share in it.

New Method for demonstrating Tubercle Bacilli on cover-glasses and in sections.<sup>\*</sup>—Dr. C. Aren's communicates the following method, which he says is quick and safe for demonstrating tubercle bacilli. It consists in staining sputum with a saturated alcoholic solution of fuchsin diluted with chloroform, and decolorizing with a solution containing hydrochloric acid (HCl, 10; aq. dest., 260; alcohol, 90 per cent., 730).

Sputum.—A fuchsin crystal the size of a millet seed is put in a watchglass together with 2 or 3 drops of absolute alcohol, or 3 drops of a saturated alcoholic solution of fuchsin may be nsed. To this solution 2-3 ccm. of chloroform are added, and when precipitation has ceased and all cloudiness disappeared, the cover-glass is stained (4-6 minutes) in the ordinary way. The chloroform is allowed to evaporate and the preparation decolorized in 96 per cent. spirit, to which 3 drops of HCl are added. It is then washed and may be examined in water straight away or be after-stained with dilute methylen-blue.

Sections.—These are removed from spirit to the chloroform-fuchsin solution and stained for 4-6 minutes, decolorized with the acidulated spirit, the acid washed out with strong spirit, and contrast-stained with dilute methylen-blue.

Influenza Bacillus, and methods for obtaining and demonstrating it.<sup>†</sup>-Herr Pfeiffer has found in the sputum of influenza, in the peribronchitic tissue and on the surface of the pleura a minute bacillus of about the thickness and half the length of the bacillus of mouse septicæmia. They were seen in the pus-cells, three or four often forming a chain. They stain with Gram's method, with the basic anilin dyes, but best with dilute Ziehl's solution or with hot Looffler's methylenblue. Pure cultivations of the bacillus were made in  $1\frac{1}{2}$  per cent. sugar-Kitasato separated this micro-organism from others mixed up in agar. the oral secretion by growing them on glycerized agar at incubation temperature, whereon they appeared as microscopic drops much like water but never running together. They were cultivated on this medium to Cultivated in bouillon white flakes appeared, these the tenth generation. sank to the bottom, leaving the fluid above quite clear, whence it was inferred that the bacillus is immobile. Inoculation experiments on apes and rabbits were successful, but on no other animals.

The same micro-organism has been demonstrated microscopically in preparations made from blood by Canon. The cover-glass preparations, having been dried in the air, were placed for 5 minutes in alcohol, and then stained for 3-5 hours at 37° C. in Czenzynke's solution (saturated aqueous solution of methylen-blue, 40 parts; 1/2 per cent. eosin solution (in 70 per cent. spirit), 20 parts; aq. destil., 40 parts); they are then washed in water, and having been dried, mounted in balsam. By this stain the blood-discs are stained red, and the white corpuscles and bacilli

\* Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 9-10.

<sup>†</sup> Deutsch. Med. Wochenschr., 1892, Nos. 2 and 3. See Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 148-50. blue. They disappear from the blood six days after the fever has passed off, and were never found in persons not sick with influenza.

Canon has also obtained eultivations of his bacillus from the blood, although their small number in the circulation enhances the difficulty of this feat. Cultivations were obtained from blood drawn from the finger-tip, and made on glycerinized agar and Petri's eapsules. The eolonies which grew at incubation temperature quite resembled those described by Kitasato.

Differentiation of Leprosy and Tubercle Bacilli.\*—It is often important for pathological purposes to know whether affections are leprous or tubercular. Dr. C. Slater finds that the distinction cannot be learnt from an examination of the bacilli, for any colouring agent which stains the leprosy bacillus also stains *B. tuberculosis*; the methods proposed to stain *B. lepræ*, while leaving *B. tuberculosis* unstained, are unreliable. Differences have been asserted to exist in the rapidity of staining and resistance to decolorization; but these are due to differences in the number of bacilli present.

We are reminded that stains coming from different manufacturers and different samples from the same maker are very variable in their staining properties. The two red dyes used by Dr. Slater were a magenta obtained frem Messrs. Martindale and a rubinfuchsine from König of Berlin.

Staining Bacteria in Fatty Substances.<sup>†</sup>—Dr. C. Arens stains bacteria in fatty substrata, e. g. milk, in the following way.

A loopful of milk and a loopful of distilled water are mixed on a eever-glass, dried and fixed by gentle heat. The cover-glass is then placed in a watch-glass containing chloroform—methylen-blue made by mixing 12-15 drops of a saturated alcohol solution of methylenblue and 3-4 ccm. of chloroform. In this solution the cover-glass is moved to and fro for 4 to 6 minutes. The chloroform is then allowed to evaperate and the preparation is washed with and examined in water. In fresh milk and cream only the bacteria are stained, but if curdled the flakes of easein are dyed pale blue, though this does not interfere with the distinctness of the deep-blue bacteria.

Staining Sections of Mosses.<sup>‡</sup>—The cortical and pericyclical zones of mosses are composed of cells so much alike that it is difficult to distinguish them nuless staining agents be used.

M. Bastit first treats them with a solution of tannin, and, after sectioning, with congo red. The tannin acts as a mordant in the cells of the pericyclical zone, these being distinguished by their brighter colour from the cells of the cortical zone. The sections are next placed in a solution of hypochlorite of soda, then of potash, by which the cell-contents are removed, and having been rapidly washed, transferred to a solution of phosphoric acid. Hercupon they become blue, but on being immersed in absolute alcohol the stain is removed except from the parts which have absorbed the taunin.

<sup>\*</sup> Quart. Journ. Mier. Sci., xxxiii. (1891) pp. 219-28.

<sup>†</sup> Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) p. 10.

<sup>‡</sup> Rev. Gén. de Bot., iii. (1891) p. 432.

Staining Motor Nerve-endings in Striated Muscle.\*-Sig. C. Negro has devised a method for simultaneously staining and fixing muscle, especially suitable for demonstrating the nerve-endings in the muscular tissue of Reptilia. The solution is made of saturated solution of ammonia-alum, 150 ccm., and saturated alcoholic solution of hæmatoxylin (Grübler) 4 ccm. This mixture is exposed to the air for eight days in an open vessel and then 25 ccm. of both glycerin and methyl alcohol are added.

The procedure is as follows. The insertion of the muscle is teased out on a slide, and when this has been sufficiently done some drops of hæmatoxylin solution are added to it. In 15 to 20 minutes it is carefully washed while on the slide and then mounted in a mixture of equal parts of glycerin and water. Put up in this way the preparation will keep for at least two years.

Another method consists in overstaining and afterwards decolorizing. This is done by immersing separated muscle fibrils in the hæmatoxylin solution for 24 to 48 hours, and after washing, to keep the preparation in the glycerin and water until required. The fibril is then teased out on a slide, and if overstained it is treated for 10 to 12 seconds with the following mixture :---Glycerin 40 parts, hydrochloric acid 1 part, distilled water 20 parts.

A too prolonged action of the acid fluid decolorizes the nerveendings and also affects their structure.

Rapid Staining of Elastic Fibres.<sup>†</sup> - Sig. E. Burci gets very by using aurantia di-trinitrophenylamine satisfactory results  $\begin{array}{c} \mathrm{C_6\ H_2\ (NO_2)_3} \\ \mathrm{C_6\ H_2\ (NO_2)_3} \end{array} \mathrm{NH}. \end{array}$ The sections, stained with carmino or hæmatoxylin, are washed in water, dipped for one or two minutes in a saturated alcoholic solution of aurantia, washed in absolute alcohol, cleared in clove oil, and mounted in balsam.

New Method of Double Staining. 1-Sig. Planese proceeds by first preparing Martinotti's solution, a saturated solution of picric acid and nigrosin in alcohol. Two parts of this solution and one part of anilin water are next mixed and allowed to evaporate in the open air. From this are deposited crystals which are dissolved in absolute alcohol. From the latter arc obtained cubical crystals of an olive green colour, soluble in water, alcohol, or ether. With these crystals are made a 2 per cent solution in spirit for tissnes, in water for micro-organisms.

The sections are first stained either with Beale's carmine or Orth's lithia carmine, and having been treated with acidulated alcohol, are washed and then dehydrated. They are now immersed from two to ten minutes in the alcoholic solution of picro-nigrosin until they assume a brown hue. They are next decolorized in an alcoholic solution of oxalic acid, dehydrated, cleared up and mounted.

By this method the nuclei are stained red, the plasma is a dark

\* Boll. dei Musei di Zool. ed Anat. Compar. della R. Unio di Torino, v. (1890). See Bull. Soc. Belge de Microscopie, xviii. (1891) pp. 9-10.

† Atti Soc. Tosc. Sci. Nat., vii. (1891) pp. 251-3. ‡ La Riforma Medica, 1890, No. 155. Sce Bull. Soc. Belge de Microscopic, xliji. (1891) pp. 13-14.

yellow, the connective tissue a pale green, elastic fibres violet, and cartilage yellow.

If it be desired to stain micro-organisms at the same time as the tissues, the sections are, after having been stained with carmine, decolorized by Gram's method, or by the Koch-Ehrlich method for tubercle bacilli, and then are immersed for five minutes in the aqueous solution of picro-nigrosin, after which the process terminates as above.

### (5) Mounting, including Slides, Preservative Fluids, &c.

Combined Method for fixing and flattening Paraffin Sections.<sup>\*</sup>— Mr. H. E. Durham has devised a modification of Canini's method in which alcohol is used as a means of eausing sections to adhere to a slide. These sections are placed on a dry slide or are moistened with ordinary methylated spirit diluted to 70 per cent. alcohol. The slide is placed on a horizontal metal plate kept sufficiently warm to soften the paraffin. More alcohol is run on by means of a pipette. As the slide becomes warm the paraffin softens, and any little wrinkles disappear, tho sections floating flat on the top of the alcohol. When they all appear flat the excess of alcohol may be removed with a pipette.

When all the alcohol has evaporated, the paraffin may be just melted and then dissolved with benzol or xylol. Canada balsam may then bo dropped on and the cover-glass applied, or the slide may be put through absolute alcohol, and stained and mounted as desired.

Care must be taken that the warm plate is not too hot, for if the paraffin melts completely the sections are unsupported. It is elaimed for the method that it is much less troublesome than Gaskell's, and quite as satisfactory for even apparently hopelessly crumpled sections.

More about Cements.<sup>†</sup>-Mr. J. D. Beck remarks :---" Invaluablo articles have been written on the nature of cements and varnishes for finishing mounts. I feel my incompetence to enter a field among superior microscopists with my suggestions, except on important points, which, in my opinion, have been overlooked, as I do not recollect to have read any comments as to the applying of anything on top of the cover-glasses of mounts. For objectives of low power this answers very well, if it is a good, hard, and elastic varnish or cement, fixing the cover-glass securely, but when it is desirable to use high power objectives of short working distance, these rings, thus applied, are in the way of the lens, which is more or less liable to injury by contact with the ring of varnish on the cover. I perceive, however, as I look over my collection of slides, that many microscopists never or seldom allow any eement or varnish to rise above the upper surface of cover-glasses. This effectually prevents all trouble with high power objectives, unless the eover is not parallel with the slide, a very common annoyance, but it does not hold the cover as securely as a thin coat of hard and elastic finishing varnish applied around the edge with a very narrow ring on top, being sure that there is no break between the ring at the edgo and that at the top.

Many coments have been prepared and are in the market which are

\* Quart. Journ. Mier. Sci., xxxiii. (1831) pp. 116-7.

† The Microscope, xi. (1891) pp. 338-41; 368-70.

very defective in preparation and formula. In a former article I recommended Winsor and Newton's picture varnish for finishing mounts. It makes a neat finish, but is not a durable coating. All the cements prepared from dammar, mastic, shellac, gum-arabic, and all the other gums or resins, to my knowledge (except copal, amber, and a resin or gum nearly as colourless and hard as glass, which resisted fifteen solvents, and for which I have not yet found a solvent or a name), all are too soft and brittle, and therefore unfit for cements or varnish.

White zinc ecment, according to my collection of mounts and all that I have purchased, and made myself, is the most defective of all cements; it is not necessary to enter into an argument, or a controversy to prove this question with any pet theories. As a practical test, 'the eating is the proof of the pudding.'

When I examine the slides received from Europe and from every State in the Union, I find that the rings of white zinc, shellac, dammar, Brunswick black, marine glue, &c., have prismatic colours between them and the slide, an evidence that the cement has cracked loose from the glass. Some of my own preparations have also cracked and curled up in course of time. My slides are in a cool room seldom heated to  $90^{\circ}$  F. in the summer, and never below  $40^{\circ}$  F., in the winter, and not exposed to sudden changes of temperature.

I have resolved to put all cements for my own use to the following practical test: spin a ring on a clean slide and let it harden thoroughly, then push a sharp-pointed seratch-awl or a sharp brad-awl through the ring, cutting a groove just wide enough for the tool to pass.

This repeated a dozen times on one ring should leave sections not less than 1/16 in. long intact, between each incision, and so hard that no impression can be made on the ring with the edge of a stout thumbnail. This I consider a reliable test. But if large sections of the ring fly away, leaving no trace of the cement on the glass when tested in this manner, as do all the white zinc cements that I have bought of the opticians, it is, without any exception, a nuisance.

It is impossible to prepare a good and reliable cement or varnish out of poor or improper materials; nor is it always possible to prepare a good article ont of the best material, if improperly proportioned, or if prepared in a hasty and careless manner.

When the best glue or gelatin is soaked in cold water all night, and then boiled in a water-bath till thin, and thoroughly dissolved by frequent stirring and by adding to it prepared chalk, chloride of sodium, glycerin (C. P.), and acetic acid in the proper proportions, we have a reliable cement for mounts and labels that will never crack nor scale off.

I have found the following original formula good; but it may be improved, I think :---

(1) Reduce 6 drm. of dry gelatin to a thin solution in distilled water—soaked overnight cold, and boiled in a water-bath.

(2) Reduce 1 drm. of prepared chalk to a thin solution in distilled water, and add to it one fluid drm. of a strong solution of chloride of sodium (half that quantity of strong alum solution, or a little chloride of calcium may be better), and stir it well; then pour it quickly into the gelatin solution; and stir the whole thoroughly, boiling it until as thick as can be poured into a bottle with a large neck.
(3) Mix one fluid dram of alcohol, 95 per cent., into a fluid dram of sulphuric ether; pour into the gelatin and mix well. If too thick, thin it with acetic acid to suit, and add 6 or 7 drops of glycerin (C. P.); mix the whole thoroughly with a clean stick.

Do not insert the cork or shake the bottle with the ether and alcohol in it, or it will generate sufficient vapour to burst the glass or blow the eork out with half the cement. When it gets too thick in a warm room through evaporation, thin with alcoholic ether if you want it to dry faster; or with acetic acid to dry slowly.

Try it on glass, and if too brittle or liable to crack, add more glycerin, say two drops at a time, mixing well and repeat if necessary, until the cement will dry hard in from two to five hours; if at the end of two weeks (in a warm room) it will bear the test referred to above, it is sufficiently hard for filling up around balsam mounts, which have become hard and solid; for labels it has no equal.

Pour some of this cement into a 1 dram phial and colour it sufficiently with a strong solution of black 'Diamond' dye, and you have a beautiful black cement. With Diamond dyes you can give it any colour. In this case, the acid must be left out as it will precipitate the dyes and spoil the beauty of the cement. Alcohol, 50 per cent., will have to be used in lieu of the acid, and the mixture placed in warm water, if too cold or thick.

By mixing plenty of moist Chinese white with the colourless cement, and grinding it thoroughly in a mortar and placing it in a bottle with a sufficient quantity of glycerin to prevent cracking, you will have a beautiful and durable white cement. On a fair trial it will be found that these cements will have no equal for durability and tenacity on glass, and that they will not run into balsam mounts if the latter are sufficiently hard for any cement which dries quickly.

It will be necessary to give the rings made of these cements several coats of amber varnish or the best copal varnish, so as to resist moisture. I do not recommend these coloured cements for aqueous mounts lest they run in. Balsam, gelatin, or gum mounts, when neatly finished with the following transparent cements, are unequalled in beauty, and probably as durable as any; they never ruin any mounts by running in, and save the time consumed in ornamenting, which really adds no essential value to slides. Procure a good colourless amber, or best colourless copal varnish, and add a little white beeswax to one bottle of amber or of copal varnish or palmitate of alumina\* instead of wax, and it will increase the tenacity and clasticity of the cements which are to be used for the body of the ring around moist or aqueous mounts, while the last, or last two coats, should be as hard as possible; they will adhere to softer coatings, while they might be too brittle to apply directly to the glass.

Good gold size has only one fault, it dries too slowly. The best copal varnishes are just as good, and dry in much less time. I abhor all fluid mounts, and therefore have no use for that miserable, brittle, crumbling white zinc cement which soon assumes a dirty mud colour.

When glass can be prepared to inclose an object in fluid and be as durable as the cells or tubes of spirit-levels, or bulbs of thermometers,

\* Dissolve in oil of turpentine.

with a vacuum chamber (for expansion and contraction) at one sido, and not interfere with objectives, then I may turn my attention to fluid media, but not till that is a success. For anhydrous cements I proceed as follows :---

(1) Give the balsam mounts a coat of good pale copal varnish as wide as the ring is to be. Good 'elastic gear varnish' is so tenacious and elastic that I have used polished steel tools (with only one coat of it) for over twenty years, it effectually protecting them from rust.

(2) Revolve the slide on the turntable and scrape or rub and polish the surface a little before applying the second coat; unless this is done the air or gloss will cause some trouble before the next coat will adhere to the surface. This operation requires care and skill.

(3) Build up or fill up the ring around the cover-glass, or, for a cell, use the same varnish with a little white becswax dissolved with it, and thinned with turpentine or benzol if too thick. Put on thin coats and give each coat plenty of time to dry and harden, so that it may be scraped or polished; it requires very little friction on the surface to make the next coat adhere, I prefer a small sharp chisel, which can be made of a bradawl or selected from engravers' tools. I have sometimes used a small stick with its end properly dressed and dipped in cold water and pulverized pumice-stone, which is then washed away, but I like the chisel best.

(4) Apply one or two coats of ivory-black mixed with a little varnish; when dry and hard, polish with a scraper, cold water and pumice stone, or any suitable polishing material.

(5) Wash with cold water and a soft brush; wipe dry with soft chamois skin or linen rag.

(6) Apply an even coat of good amber or copal varnish. I find good copal varnish, called 'elastic gear varnish,' and used on carriage gearing, better than any gold size. It has to go through mud, rain, sand, the burning sun, expansion of heat and contraction of cold, on carriages and railroad passenger cars. The body of the ring should be hard, solid, tough, elastic, and above all, devoid of brittleness; it should be built up to the top of the cover-glass and finished with the best amber or copal varnish.

To make a colourless copal varnish, select the palest lumps of copal gum and crush them into small pieces, but do not pulverize when full of dirt; tie in a bag of fine muslin, and suspend in a widemouthed bottle of sulphuric ether, when the copal will gradually ooze out into the ether. When the gum has been digested, let the bag drain off and be thrown into another bottle of ether, which will remove all the available gum. It is a good plan to have plenty of the gum so that the liquid will form a varnish sufficiently thick. Then add oil of caraway or any slow drying essential oil, as oil of anise, or poppy, or sweet almonds, which are as colourless as possible in such small quantities; this will make the varnish dry more slowly and render it more elastic. When it dries properly, yet is too thick, add oil of rosemary or some such colourless essential oil that it may dry about as fast as it may be required. If it should dry too slowly add more ether and mix thoroughly.

Some of the essential oils, although colourless, havo slightly

296

coloured my varnish, yet it is the most colourless I ever saw. It is elastic, drics hard and endures the test admirably, and is very tenacious; it is easily prepared in the way described."

#### (6) Miscellaneous.

Qualitative and Quantitative Microbiochemical Analysis.\*—By microbiochemical analysis, M. W. Beycrinck means the employing of micro-organisms for demonstrating the presence and amount of certain substances of fixed composition, and the intention seems to be to obtain evidence of the nature of organic fluids from the extract of plants and from the products resulting from the action of forments; and further to ascertain the constituents of very dilute solutions which are suitable for microbic growth.

For the qualitative analysis the auxanographic method † may be employed with advantage. The quantitative method depends, in principle, on the transference of the element or compound to the microbic substance and the quantitative determination of the latter by the enumeration of the colonics.

It would appear that this method is intended to determine the quantity of organic matter in dilute solutions and in drinking water by the growth of micro-organisms, and also to ascertain the total amount of nitrogen. It is stated that this method is extraordinarily sensitive, but the working details are not forthcoming, and without these a new method of procedure is difficult to follow.

Demonstration of Starch and Cellulose.<sup>‡</sup>—Prof. M. Hönig recommends the following process for the demonstration of the presence of stareh and cellulose, and for the separation of these two substances from one another and from albuminoids. If a mixture of cellulose, starch, sugar, and albuminoids is heated with glycerin to 210° C., the cellulose undergoes no change, while the starch is transformed into a mixture of soluble stareh and dextrine, which dissolves completely in hot water into a limpid fluid, and can be again precipitated by a mixture of 5 parts alcohol and 1 part ether, and the amount determined. The sugar and albuminoids are dissolved, and are not again precipitated by ether and alcohol. A practical method is described, founded on this reaction, for the determination of cellulose and starch in fibres.

The Leeuwenhoek Microscopical Club.§ — The members of this private club, at Manchester, have published the records of their proceedings from 1867 to 1891. They have found that, for their purposes, six or eight persons form the most convenient numbers for effective consideration and discussion. The club has always used the Microscope as an accessory to investigation.

\* Centralbl. f. Bakteriol. u. Parasitenk., x. (1891) pp. 723-7.

† See this Journal, 1891, p. 800.

‡ Verhandl. Naturf. Ver. Brünn, xxix. 1890 (1891) pp. 23-5.

§ 'A Review of the Work of the Leeuwenhock Microscopical Club, Manchester, 1867-91.'

aided by bromide paper enlargements from photomicrographic negatives, turned the scales and won a decision in favour of the plaintiff.

# Photographs of Photomicrographic Apparatus.

In the fifth edition (1880) of Dr. Beale's 'How to Work with the Microscope,' p. 309, will be found the woodcut seen in slide 50, representing my wet plate apparatus as it stood at that time. The old support, consisting of a camera bed about six feet long with a single leg at one end and a round table-top expansion with three legs at the other, is also the support of my present apparatus. The sliding-box camera has been replaced by a bellows camera taking plates up to 8 by 10 in. The special Microscope has been replaced by a Powell and Lealand No. 3 stand, which with accessories rests on the table-top expansion.

Slides 51 and 52 (figs. 35 and 36) show the general arrangement of the apparatus on the table-top expansion. The camera front moves freely toward the Microscope, a hole in the front board receiving the eye end of the horizontal Microscope-tube until the board comes in contact with a black card fitting light-tight about the tube. The camera front is easily removed, so the eye can glance through the Microscope without changing the position of the instrument, or, so the Microscope and all the apparatus for artificial lighting can be turned to one side for arrangement and then turned back again. That the latter may be done, the Microscope and lighting apparatus stand upon a board which revolves about a vertical axis passing through the object on the stage. A similar convenience is familiar to you in Mr. Pringle's apparatus.\* I prefer, however, to have the Microscope and camera constantly connected and to use a secondary horizontal tube, at a convenient height for the eye when sitting, projecting at an angle of 90° from one side of the ordinary axial tube. A plane mirror silvered and polished on its objective surface and set at an angle of  $45^{\circ}$  reflects the picture forming rays from the axial into the side tube. The eye-piece of the side tube is 10 in. (central measurement through the two tubes) from the objective. When the position of the object and its illumination are satisfactory, the mirror is withdrawn wholly into the side tube, allowing the objective to project an image into the camera. The axial tube, in addition to having its inner surface well blackened, contains a sufficient number of diaphragms to thoroughly prevent internal reflection. When an eye-piece is not used in the axial tube, a dummy eye-piece, with diaphragms, but without lenses, is inserted to prevent reflection from the interior of the eye end of the tube, where it is always more or less bright. I have found this dummy eye-piece a matter of importance. I believe the

\* This Journal, 1890, pp. 513-7, 666.

bright surface it should cover has been quite commonly neglected and has been the cause of many poor results in photomicrography.



In slide 52 (fig. 36) is seen a block of heavy wood which slides in a direction parallel with the axis of the Microscope, without lateral

FIG. 35.

movement and on cloth bearings, between two cleats of hard wood screwed to the table-top expansion. The block is moved back and



forth by a brass rod supported all along one side of the camera bed. From the top of the block projects towards the Microscope a small bar of wood with a screw-eye at its free end. A lever-rod fastened by a ring and milled-headed screw to the coarse-adjustment wheel drops into the screw-eye. By this simple contrivance coarse focusing can be done on the ground glass at any distance from the Microscope by moving the long brass rod in its support, and so moving the block and lever. From about the fine-adjustment wheel (fig. 35), an endless cord passes down and about a pulley-wheel, actuated by a brass rod, supported all along under the camera bed, so that fine focusing can be done at any distance from the Microscope. Coarse focusing is done on the ground-glass, fine focusing by a focusing lens held against a piece of polished plate-glass substituted for the ground-glass.

Projecting from the sliding block is a second bar ending with a screw-eye which receives a lever from the rack and pinion moving the draw-tube.

Either lever is quickly fastened or loosened by a thumb-screw closing or opening the ring about the milled wheel. First one lever alone, or the other, is used; never the two together. The draw-tube lever is used when by the Woodward method \* the back focus of an objective is extended by an achromatic meniscus screwed into the objective end of the draw-tube. It seems to be almost forgotten that Dr. Woodward produced his famous photomicrographs by this method. The method, briefly, is first to arrange the object and illumination, to adjust the objective-collar most carefully for the 10-in. distance, then to remove the eye-piece, to screw the meniscus into the objective end of the draw-tube, and, finally, to adjust the draw-tube until the meniscus is at the right distance from the back of the objective to extend the focus to the ground glass, the objective meantime remaining undisturbed in its best condition. No other method gave as good results until the apochromatic method was introduced. In the apochromatic method, however, the objective is not left undisturbed, and the projecting eye-piece has two lenses with four reflecting surfaces. If it were practicable to make a compensating concave with only two reflecting surfaces, analogous to the Woodward meniscus, to be used instead of the projecting eye-piece, a so modified apochromatic method would be more rapid and might perhaps give even more brilliant results.

The lamp, edge of the flame on, and bull's-eye are carefully centered on sliding boards, so as to be able conveniently to use Mr. Nelson's method of illumination.<sup>†</sup> The light (fig. 35) passes through an alum or water cell, and an ammonio-sulphate of copper cell —seen between the bull's-eye and substage condenser—to eliminate most of the heat-rays, to give visually monochromatic light, and to make the visual image approximately the same as the actinic image. I believe by using the blue cell I have been able to approximately focus the actinic rays; for I have used very various objectives with the blue

\* This Journal, 1879, p. 664.

† Op. cit., 1885, p. 713.

cell and have not once noticed a want of coincidence of visual and actinic foci. Further, I have focused with lamplight not passing through the blue cell, and then photographed with the blue cell in place with a result quite inferior to the next, the next being obtained under the same conditions excepting that the blue cell was in place when focusing. The use of the blue cell in focusing secures excellent results with the Wales photo-objectives, corrected for the violet ray. After passing through the blue cell the light is received by a substage condenser and focused on the object. My substage not only carries ordinary Powell and Lealand achromatic condensers for high power work, but by means of adapters also carries, instead, an eye-piece or a suitable objective as a condenser for low-power work.

The round table-top expansion (figs. 35–37) is divided by degree markings, enabling me to turn the board supporting the lighting



apparatus to any required angle for oblique illumination of transparent objects, or so far around as to cause the light to fall at suitable angles on opaque objects. That end of the revolving board which is under the Microscope is circular. The upper surface of the circular portion presents two levels, a central circular higher level surrounded by a peripheral lower level. On the latter rest the three feet of the Microscope tripod, in such fashion that when the two feet away from the camera, or under the substage, are snugly against the edge of the central raised level, the object on the stage is in the vertical axis about which the board revolves. Therefore, after the board has been turned out of line for oblique illumination, the Microscope can be placed in new correct position immediately, by bringing the two feet mentioned against the edge of the higher level, and the eye end of the axial tube to the centre of the camera front. In photomicrographing an opaque object, a diaphragm cap is slipped over the front of the Thus a diaphragm comes to be supported about half-way objective. between the object and the objective : it there cuts off all rays from the object or stage outside the area to be photographed, and is analogous to the hood of a portrait or landscape lens. When an objective has a short working distance, a piece of dead black card or paper with a central hole, instead of the cap, covers all the object except the area to be photographed. In photomicrography, as in all critical optical work, it is important here and everywhere to shut out or suppress, so far as possible, every non-effective or wandering ray of light. The wainscoting of my room is black; the woodwork of the apparatus is black; the walls and floor are non-actinic; by day I always photomicrograph with covered windows; and at night I have no light in the room but that on the revolving board.

In slide 52 (fig. 36) is also seen a small cubical box projecting from the front board of the camera. Within the box is a mirror which reflects the light from a white card, substituted for the groundglass, through a single opera-glass fastened to the nearer side of the box. With this addition to the apparatus in place, I can sit at the side of the table, arrange the object and illumination with the eye at the side tube of the Microscope, withdraw the mirror from the axial tube and then, without getting up, look through the opera-glass and roughly focus an image on the white card. Focusing is then completed at the other end of the camera in the usual way by means of the fine-adjustment rod and pulley. At the left are seen two screwclamps which are used in fixing the camera back at any required distance from the Microscope, the exact distance being shown by a scale cut on one side of the camera bed. The eye end of the tube is seen to be supported by an adjustable standard, a necessity in delicate work with high powers.

Slide 59 (fig. 38) shows an arrangement of apparatus for photomicrography with transmitted axial sunlight. To the left is an upright supporting a horizontal axial tube with a portion of its upper half cut away to allow an alum or water cell, an ammonio-sulphate of copper cell, a disc of ground glass, and diaphragms, one or more of these, to stand in the lower half. The Microscope is seen to the right. Between is an 8-in. portrait lens so supported by an upright that its distance from the Microscope can be varied at pleasure, by sliding the upright along the revolving board. The lens is attached to the upright by two sliding boards, one sliding vertically and one horizontally, for the purpose of centering. The portrait lens was originally intended to eliminate diffraction phenomena in accordance with the Woodward method; \* but the lens is also occasionaly used instead of a substage condenser. Outside a south window, slide 57 (fig. 39), is a Stratton

\* Monthly Microscopical Journal, vi. (1871) p. 170.





and Burrill heliostat \* on a shelf-table. The window-sash is glazed with plate glass through which the light from the mirror passes without that deterioration which occurs when light passes through the wavy surfaces of an ordinary pane. The place for the heliostat is so marked that it can be within a minute accurately set and levelled over a meridian line cut in the surface of the shelf-table. I have neglected to say previously that the whole apparatus in the room is also accurately placed and levelled over an extension of the same meridian line cut in the floor. By referring to figs. 35 and 36 it will be seen that the table-top expansion is not directly supported by the three legs. The three legs support a ring into the lumen of which fits nicely but freely a circular plate of hard wood fastened centrally to and below the table-top expansion. This construction permits the camera and Microscope to be turned out of meridian while the revolving board remains in meridian for oblique On returning the camera support to the illumination with sunlight. meridian, cleats on the floor stop the instrument in correct position. In photomicrographing opaque objects by sunlight the heliostat is shifted to a meridian one foot to the east, as are also the alum and ammonio-sulphate of copper cells. The sunlight from the heliostat, after passing through the cells, is received by a long-focus concave mirror and reflected on the opaque object.

Slide 55 (fig. 40) is a photograph of a Powell and Lealand No. 3 stand with a side tube added for photomicrography and a lever attachment to the pinion of the draw-tube. The mirror, now in the side tube, can be moved into and out of the axial tube by means of the milled-headed screw seen under the side tube. It is obvious, too, that the use of the tubes can be reversed : the Microscope can be used in the vertical position, the object and illumination arranged in the ordinary way, then the mirror pushed into the axial tube to reflect the image-forming rays into a camera attached to the side tube. To the right is seen the adjustable standard for supporting the eye-end of the axial tube when horizontal. It is adjustable in height by means of the milled-head screw arrangement seen about one-fourth down the standard.

A convenience peculiar to the Powell and Lealand, or Ross, model of stand is shown in slides 61 and 62 (not figured). The axial tube is removed and its supporting arm turned to one side. By means of stage forceps various large objects are supported, or on a broad wood superstage a bit of manuscript is flatly fastened, while a short-focus doublet landscape lens on the camera front serves, instead of a Microscope objective, to cover a comparatively extensive area and give a perfectly flat field.

Exposures are made in a very simple way. While the sensitive plate is being placed, a black card stands close to the ammonio-

\* Proc. Amer. Soc. Micr., 1885, p. 103.

sulphate of copper cell and intercepts the light. When the plate is in position and all the apparatus satisfactory, the light-intercepting card is removed for the required length of time and then replaced.



In bringing this paper to a close, I wish to acknowledge my indebtedness to Mr. Edward Bausch, of the house of Bausch and Lomb, for practically placing the resources of the firm's great factory at my disposal in the construction of the more special and fine brasswork so necessarily a part of the apparatus.

## MICROSCOPY.

## a. Instruments, Accessories, &c.\*

(1) Stands.

A new Construction for the Microscope.<sup>†</sup>-Dr. A. Lendl remarks that in the use of the Microscope the chief consideration has hitherto been rightly paid to pure definition. The latest excellent objectives and immersion systems leave little to be desired in this respect, so that it is now time to seek to combine with this improved power of definition a much increased magnifying power.

The author attempts to effect this independently of the objective and without increasing the magnifying power of the eye-piece by a



change in the construction of the Microscope. The eye-piece is removed and replaced by a second complete Microscope, so that the image formed by the objective is no longer observed by the eye-piece but by

\* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous. † Zeitschr. f. Wiss. Mikr., viii. (1891) pp. 281-90.

## 414 SUMMARY OF OURRENT RESEARCHES RELATING TO

this auxiliary instrument. The author claims that by this means the resolving power and definition are not injuriously affected, and that the



field of view is not so much darkened as by the use of stronger eyepieces. In his experiments the author used a large Reichert Microscope. In this instrument on the upper end of the body-tube a ring (fig. 43) is screwed. In this is fitted a tube which carries the eye-piece and can be introduced and removed at will. For use with the auxiliary apparatus the ring is made to slide into instead of being screwed on the body-tube (fig. 44). It can thus be easily removed together with the tube carrying the eye-piece, and replaced by a longer tube containing at the lower end a condensing lens, and at the upper the auxiliary Microscope which is adjustable by rack and pinion (fig. 45).

The complete apparatus is represented in fig. 46.

It is not intended to make use of these very strong magnifications for continuous work, but only for the closer examination of details left in doubt by the ordinary method of observation. For such purposes the diminution of the field of view is no disadvantage.

As source of light the author uses the Koch-Wolz Microscope lamp combined with the Auer incandescent light, and also the electric incandescent light. The last allows of a magnification of from 8000 to 10,000 times.

The author has applied his method of observation in order to decide the vexed question of the shape of the pearls of *Pleurosigma angulatum*. With a Reichert homogeneous-immersion 1/20 and strong eyc-piece No. V. the pearls give their usual hexagonal appearance, but by the use of the auxiliary Microscope with objective No. 2 and eyc-piece I. or II., they are seen to be unmistakably rhombic in outline. The upper surface is curved and the edges and corners are truncated. The acute angles of the rhomb are rounded, so that on account of the deeper shadow, under lower magnification they give the appearance of two parallel sides of the hexagon. In some parts of the valve the pearls had disappeared, leaving however the very fine membrane on which they rested. This membrane was found to have kept the impression of the pearls as a rhombic pattern.

Surirella gemma was also examined. The striations are composed of small particles which give rise to the so-called "basket-like network." Each row appears like a string of pearls.\* Here again in many cases pearls have disappeared, leaving a membrane marked by very fine striations. In the perfect specimen this striation does not coincide with, but is covered by the lines of the "basket-like network." With extremely high magnification, however, and by careful adjustment, it is possible to see first the network and then the striation. The combined images give the false appearance of elongated hexagons.

Zentmayer's Dissecting Microscope.<sup>†</sup>—This instrument, made primarily for botanical work, has a circular base 5 in. in diameter, and is made of polished brass. A stout pillar rises about 6 in. on one side, to which a broad stage (4 in. by 5) and a jointed arm for carrying the lenses are attached. A plane mirror is adjusted to the base beneath the stage. The latter carries spring clips, which are easily removed to

† Amer. Mon. Micr. Journ., xiii. (1892) p. 2.

<sup>\*</sup> See this Journal, 1890, Plate II.

make room for a glass plate which fits the well of the stage. With the instrument come two lenses, 1 in. and 3/4 in., which may be combined



to secure a 2/3-in. focus. The plan of this Microscope was suggested by Prof. J. T. Rothrock, of the University of Pennsylvania, and it is used in his botanical classes.

#### (2) Eye-pieces and Objectives.

Apochromatics.—Mr. Edward M. Nelson writes to us: —"In these days it becomes an important question whether a given objective contains fluorite or not. This can be easily determined by means of the polariscope. Applying this test, I find that in the 24-mm. objective it is the middle combination; in the 12-mm. it is the back, and the combination immediately behind the front lens; in the 1/4 and in Powell's apochromatic condenser it is the middle and back combinations which have spar in them."

## (3) Illuminating and other Apparatus.

Stratton's Illuminator.\*-This lamp is recommended as an addition to the paraphernalia of a microscopist. The supporting rods are con-

\* Amer. Mon. Micr. Journ., xiii. (1892) pp. 1-2.

nected by stiff joints, which cause the lamp to remain in any position in which it has been placed, and the possible positions of elevation or of obliquity are such as to give light in any manner desired.

The oil-tank can be rotated in the holder, thus turning the edge or the broadside of the flame towards the Microscope, as necessity may

require. The thick glass chimney as well as the flame is concealed by the metallic hood, well blackened, while a projection on the side of the hood directs the rays of light towards the bull's-eye. The latter, of unusual size (3 in. diam.), is supported by an independent support. The advantage of this is that the entire illuminating apparatus may be disconnected from the bull's-eve, or the light directed otherwise than through the bull's- . eye if more general illumination is desired. The light from the flame is passed through a ground glass or blue glass at pleasure, and before reaching the bull's-eye. Strong or faint light, direct or oblique light, can all be had easily and without changing any adjustment of the Microscope. The 1/2-in. wick gives a very good flame, kerosene being used to burn. The arrangements for draught and for cleaning are good.



Some new Improvements applied to the mechanical part of the Microscope.<sup>\*</sup>—M. Yves Delage, in collaboration with M. Nachet, has devised several improvements in the centering and other mechanical arrangements of the Microscope. In their new triple nose-piece or revolver the spring-catch consists of a cylindrical roller C (fig. 49), which is carried by a powerful spring R attached to the fixed piece of the revolver. This spring serves to press the roller into a semi-cylindrical groove hollowed in each of the arms of the movable piece. The groove, which has a radius a little less than that of the roller, so as to prevent all lateral motion, is cut in a small sliding carrier, which is kept pressed against a powerful spring by the conical serew-head F. By turning the serew the carrier is moved and the point of the movable piece displaced until arrested in front of the fixed roller. By this means the transverse adjustment is effected.

For the adjustment from front to back a movement of rotation about a horizontal axis is employed. For this purpose the central part of the movable piece is formed of strong tempered steel, flexible enough to yield beneath the pressure of a screw, but sufficiently rigid not to

\* Arch. Zool. Expér. et Gen., x. (1892) pp. i.-ix.

1892.

vibrate. A screw V traversing obliquely the shoulder of the movable branch, presses on the terminal part, and bending the steel plate raises the objective, which thus describes a small circular arc from front to back.

Calculation shows that the harmful effect on the image of this angular displacement is quite insignificant. Thus, let l be the vertical distance between the axis of rotation and the object under examination, L the



distance of the axis of rotation to the real image of the object formed by the objective, d the distance of the object to the axis, and D the distance of the real image to the axis. Then d = Dl/L. But l is approximately the length of the objective for all high magnifications; it is equal to about 30 mm. for a homogeneous-immersion objective; L is the distance of the base of the objective to the diaphragm of the eye-piece, i. e. about 150 mm. The ratio l/L is therefore about 1/5. At the most, D rarely exceeds 1 mm, when the objectives are screwed directly on the body-tube. This gives to d a value of 1/5 mm. On the other side, if a denote the angle through which the axis of the objective has turned, we have

$$\tan a = \frac{d}{l} = \frac{1}{150} = 0.00566,$$

which gives for a a value less than  $0^{\circ}$  23'.

The condenser (fig. 50) is movable on two rectangular axes, which allow of its adjustment in all directions in a horizontal plane. For this



purpose it is fixed on a small sliding earrier, and a serew B' regulates the adjustment in the transversal direction. For the adjustment in the 2  $\oplus$  2 direction at right angles, the movement of rotation by which the condenser in the older instruments could be drawn out from beneath the stage is utilized. The only addition is a screw B which works against a fixed projecting piece beneath the stage. This movement is, of course, not rectilinear, but on a circumference of 5 cm. radius an arc of 1 to 2 mm. coincides practically with its tangent. The vertical movement of the condenser with its iris-diaphragm is effected by a lever L, which acts on a carrier supporting the entire piece. The author considers the usual slow movement by micrometer-screw to be an absolutely superfluous complication.

The iris-diaphragm (fig. 50), which is fixed beneath the condenser at an invariable distance from the lower lens, is mounted like the condenser, except that the central position is determined by a catch. This central position, however, can be passed in either direction so as to obtain the effects of oblique light. A circular movement allows the obliquity to be directed in all azimuths.

In the adjustment of the instrument the invariable axis of the bodytube is taken as the basis from which to start. With this object, the Microscope, armed with eye-piece and objective screwed directly on the body-tube, is directed upon a cross-wire which is brought into the centre of the field by means of the movable stage described below. The condenser is then adjusted by centering either the summit of its luminous cone or the aperture of a small diaphragm above its upper lens. The iris-diaphragm is similarly adjusted, while it is opened just wide enough



to give the diameter of the field. The triple nose-piece with its objectives is then attached, and each arm is separately adjusted by observation of the aperture of the small diaphragm above the condenser which is brought to the level of the stage. For this purpose the observer, without removing the eye from the eye-piece, first works the screw F until the aperture is brought on to the axis of the field from front to back, and then the screw V until it is brought on to the transverse axis.

The movable stage (figs. 50 and 51), designed by the author, is intended to remedy some of the inconveniences of the ordinary apparatus. It possesses three advantages over the ordinary stage: (1) it can be attached and removed in a few seconds; (2) it is movable in the transverse direction and from back to front; (3) it can be adjusted by one hand only. It has the following arrangement :- The fixed stage is traversed in the middle from back to front by a rectangular slit V (fig. 50), about 1 cm. wide, which extends from the column of the slow movement up to about 1 cm. from the aperture of the stage. In this slit is a horizontal micrometer screw V (fig. 51), which is provided on the extremity at the back with a vertical-toothed wheel P, working in a horizontal toothed wheel R, which is mounted on the column of the slow movement. The wheel R effects the movement of the micrometer screw V, while the latter acts on a small carrier C, which is flush with the stage, and, guided by the bevelled edges of the slit, traverses it from back to front. The movable stage is attached to this carrier by four vertical pins which fit into four corresponding holes in the carrier. It consists of a carrier movable in the transversal direction by means of a long micrometer screw terminated at each extremity by a screw-head P (fig. 50). Attached to the carrier in front is a plate M (fig. 51) cut away considerably in the centre in order not to hide the aperture of the fixed stage in any of its positions. This plate slides on two blunt ivory points, and the preparation is held firmly upon it by two small clips.

In the adjustment of the stage, the lateral movement is effected by the screw-head P on the right, which is held between the thumb and first finger of the right hand, while the movement from back to front is produced by the simple pressure on the wheel R of the thumb, which can be bent back without displacing the hand.

The two carriers are provided with scales graduated in millimetres, with verniers reading to 1/10 mm.

The slide containing the preparation under examination has its position fixed by being fitted into a sort of rectangular box in which only two sides are retained, viz. the back and left.

The transverse displacement is not of sufficient extent to allow of the examination of the whole length of a slide. To meet the case, however, when the preparation happens to be mounted at the end of the slide, the back edge of the rectangular box which holds the slide is dovetailed in a sliding piece, so that it can be displaced laterally by simple pressure of the fingers, and take three positions marked by a catch.

The author mentions, in conclusion, the Nachet eye-piece with very large field, and a convenient accessory to this in the shape of a small hood which can be placed over it. It is provided with a deep conical groove in which there fits a piece of black card, so cut as to protect the eye from external rays of light.

A new Fine-adjustment for the Substage.—Mr. G. C. Karop writes to us:—Every microscopist who works with modern high powers of large N.A. in combination with wide-angled condensers, soon becomes aware that the usual focusing arrangements of the substage are not sufficiently delicate; for it is necessary, if the best possible resolutions are required, that the image of the flame given by these condensers, whether dry or immersion, should be as accurately adjusted in the focal plane as the object itself. Although this is well understood, there are, so far as I am aware, but few devices for the purpose of obtaining this nicety of adjustment: Mr. Nelson's, a cone actuated by a milled head on the left, and pushing against a spring, fitted only to a few No. 1 Powell stands; a micrometer movement with the milled head at the end of the tail-piece, in the large Baker-Nelson model; and Messrs. Watson's, a



their well-known "Climax" fine-adjustment to the slide carrying the substage, but it is actuated by a milled head borne on the spindle to



lever movement with the head placed at the upper right-hand side of the main stage, in their Van Henrck instrument. Working as I do with one of Messrs. Swifts' stands, I suggested to them the advisability of providing a fine substage movement for their larger instruments, and they have carried out and improved upon my ideas with their usual mechanical skill. The movement essentially consists in the adaptation of

which is connected the pinion of the coarse or rack motion; in fact, so far as the arrangement of the two milled heads is concerned, it resembles the "Tnrrell" stage movement. One complete turn of the inner head raises or depresses the substage the 1/125 in., so that very small fractions of this amount are readily obtainable, and as the two movements are close together and move in the same direction, manipulation is very easy. The "lift" is as nearly as possible central.

I submit this adjustment with confidence to those microscopists

who have felt the want of some such contrivance when working with the highest class of optical appliances.

The Camera Obscura v. the Camera Lucida.\*—Dr. Henry G. Piffard remarks:—"In drawing from the Microscope three methods are in vogue: (1) The observer studies the subject on the slide, and when he thinks he has the outlines and details sufficiently impressed on his mind, withdraws his eyes from the tube and commits the mental pieture to paper, using both eyes in directing the movements of the pencil. (2) The observer, looking down the tube with one eye (usually the left), is enabled to see the virtual image by a sort of autoprojection delineated

\* The Microscope, xii. (1892) pp. 92-3.

on a piece of white paper by the side of the Microscope. With the free right eye he guides the pencil in tracing on the paper the magnified map of the object. (3) In using the camera lucida a single eye is used for observing the object on the slide, as well as for guiding the pencil in tracing its reflected image. Method 1 requires a good memory and considerable skill as a draughtsman. No. 2, less skill is required, but the knack of doing it can only be obtained by practice. With No. 3, reasonably normal vision is a pre-requisite. The writer has for a long time, perhaps always, been affected with astignatism and hypermetropia, to which advancing years have added presbyopia, and in consequence is unable to use the camera lucida with satisfaction. In No. 1, binocular vision is employed in the operation of drawing; in No. 2, monocular vision; and in 3, semi-ocular vision.

The inconveniences referred to may be avoided by a simple device, which the writer has of late used with some satisfaction, namely, a rightangled prism with silvered hypothenuse. This should be mounted with a short tube extending from one of the square surfaces, and of suitable size to enter the tube of the Microscope. A similar short tube of a size to receive the ordinary eyc-piece extends from the other square face of the prism. If now the Microscope be placed with the tube horizontal and the prism case with eye-piece be inserted, the ocular pointed downward, an image of the object on the stage will be projected on a piece of drawing paper beneath, provided of course that there is sufficient illumination beyond the stage, and that no light reaches the paper except that coming through the objective. Personally I find this instrument much more convenient and satisfactory than the camera lucida. Mechanical micrograms must yield, however, to photographs; and the micrographic science of the future will seek the aid of the pencil less, and make more frequent use of the convenience and accuracy of photography. Bausch & Lomb made and mounted for me the prism described, and I have no doubt will be pleased to duplicate it for others."

KIRSCHMANN, A — Ueber die Herstellung monochromatischen Lichtes. (On the Production of Monochromatic Light.)

Wundt, Philos. Stud., 1896. Zeitschr. f. Wiss. Mikr., VIII. (1891) p. 420.

#### (4) Photomicrography.

Text-book of Photomicrography.\*—The aim of the author in this text-book has been to give an account of the more important contributions to the now rapidly increasing literature of photomicrography. Nothing in this direction has been attempted since the appearance of Moitessier's excellent work in 1866. The author traces out the historical development of the apparatus and methods of photomicrography, and shows how the processes now in use were gradually perfected. The book is divided into eight sections. The first treats of the apparatus required for photomicrography from the general point of view of its production and adjustment; while the second deals with optical questions, and gives a history of the gradual development of objectives and eye-pieces. In the third section the various sources of light are described, and the

\* 'Lehrbuch der Mikrophotographie,' by Dr. Richard Neuhaus, Braunschweig. See Central-Ztg. f. Optik u. Mechanik, xii. (1891) p. 282. influence of the wave-length on the resolving power of the apparatus is discussed. The fourth section deals with the development of the methods of illumination by reflected and transmitted light. The other sections are devoted to the improvements in negative and positive processes, treatment of preparations, &c. The book is illustrated with 61 woodeuts, four autotypes, two photographic plates, and one photogravure.

Photomicrography of the Solar Spectrum and Absorption Spectra.\* —D. Joaquin Ma. de Castellarnau describes the method which he employs to obtain photomicrograms of the solar spectrum. In the disposition of the Microscope and spectral eye-piece which he adopts, a ray of sunlight is directed by a Prazmowski heliostat upon a mirror by which it is reflected along the axis of the Microscope placed in the horizontal position. The Abbe condenser projects an image of the sun upon the plane of the stage; the objective and field lens of the eye-piece form a



second image in the plane of the diaphragm, and lastly the front lens of the eye-piece produces a third image on the ground glass of the camera. Between the front lens and camera is interposed a direct vision spectroscope. The eyepiece employed was a special projection one of Zeiss which consisted of two lenses giving a system aplanatie and perfectly achromatie. For taking absorption spectra the transparent solid or liquid under examination is placed on the stage of the Mieroseope.

Portable Heliostat for PhotomicrographicWork. —This heliostat was exhibited by Mr. C. Lees Curtics, at the meeting of November 18th last. It is made in the form suggested by Mr. T. Comber, and eonsists of a heavy

brass base with levelling serews and spirit-level. A clock, rotating a spindle once in twenty-four hours, is hinged to the base. This spindle can be set to suit the latitude of any place between  $15^{\circ}$  and  $70^{\circ}$ , and earries a small adjustable first surface mirror. A second similar mirror, but larger, on an adjustable stand, is also fixed to the base, and if set at half the angle of latitude of the place, reflects a horizontal beam

<sup>\*</sup> Cronica Scientifica, Barcelona, August 1889, 7 pp. (sep. copy).

of the sun's image received from the small mirror in a northerly direction (fig. 54).

The mode of using the instrument is as follows:—Set the base as marked to the true north, level base, set small mirror to latitude as engraved on the arc at side of clock, and adjust this mirror to throw an image of the sun upon the centre of the larger mirror; if the photomicrographic apparatus be also placed due north and south, this image will be reflected through the Microscope to the focusing screen of camera or sensitized plate, whichever is in position.

Brass caps to protect the mirrors are provided.

**Photomicrography of Podura-scales.**—The following is the substance of the Hon. J. G. P. Vereker's remarks when exhibiting his preparations at the meeting of the Society on December 16th, 1891:— "I have been lately experimenting in photomicrography upon some scales of *Podura*, and have obtained results which seem to throw some light upon the structure of this difficult object.

The prints exhibited are from the negatives which I have obtained, and they appear to prove that the *Podura*-scale consists of a hyaline beaded membrane, having minute featherlets inserted in it as described by Dr. Edmunds in his paper to your Society.\*

The photograph taken by Zeiss 4 mm. apochromatic lens gives a magnification of 480 diameters, and shows two different resolutions, one the well-known "exclamation" marks, and the other the scale covered with minute featherlets, which seem to stand out above the surface; it was on this latter resolution that the final focusing was done. To test this resolution, I took a photograph of a scale with dark-ground resolution, using for this purpose an immersion paraboloid, as shown to me and others by Dr. Edmunds, some years ago. For this purpose I used a water-immersion of Nachet's No. 9 (about 1/13 in.), and this photograph seems clearly to show that they stand out above the basement as minute featherlets with a forked end, There is an indication of markings on their surface and a central midrib appears in some of them. I have tried to get further resolution of these featherlets by means of a 1/12 in. oil-immersion of Reichert's, but have failed, though tho appearances would seem to indicate markings like a simplified Lepisma scale.

The standing up of the featherlets, as we may call them, is well shown under dark-ground illumination, as in this method the scale itself acts as the light radiant, showing the scale brightly illuminated on a black ground.

I also exhibit a photograph of a fine scale of speckled *Podura*, kindly lent to me for this purpose by Dr. Edmunds; it is taken by direct illumination with a 1/12-in. of Reichert's, N.A. 1.25, and is magnified about 900 diameters. It shows the beaded appearance of the hyaline base membrane of the scale. These markings exist in both *Podura plumbea* and speckled *Podura*, but are more strongly developed in the latter scale. The structure of the base membrane seems to be such that at the broadest part of the scale there are one or two rows of beads between the featherlets, whilst towards the base and top of the

\* This Journal, xviii. (1877) p. 85.

scale the beads rather tend to form single rows. In many places the beads seem to be oval, but this I hold only to show that the beads are not properly separated. In the photograph the change from the oval to the double bead is easily traced, but whether this is optical or physical I am unable to say positively."

Mr. Vereker writes to say, under date March 15th, 1892, "I regret that I was away in the north of England when my paper on the *Podura*scale was read, but if you will allow me I should like to answer one or two points in the discussion which followed.

I used a water-immersion of Nachet's, No. 9, which is nominally a 1/14, but really I think a 1/13, for the resolution with the immersion paraboloid, as owing to its great extent of correction I was able to work on a dry slide; and also because my 1/12 oil-immersion of Reichert s would not work satisfactorily on a dry slide. I may say, however, for those who wish to nse an oil-immersion, that Powell and Lealand's new apochromatic oil-immersion will work on a dry slide, and I inclose you a photograph of *Podura plumbea* on dark ground done with it. On the other hand I am doubtful whether there is much gain in so using an oil-immersion. As for isolated featherlets, I have just got a photograph with some, and with all due deference to others, there are featherlets missing on the scales on the slide; but I think, owing to the thickness of the pile in some places, it would be very hard to find out where a solitary featherlet was missing.

In reference to the beaded structure, it was photographed with direct light, and on the focusing screen the beads with Reichert's oil 1/12 stand out very clearly against the rest of the scale; in the slide I used they were quite distinct from the featherlets, which indeed are not in focus at the same time as the beaded structure. The scale used was a specially selected one kindly lent by Dr. Edmunds and by a well-known preparer."

A simple Apparatus for Photomicrography.\*—Mr. T. H. Mnras describes an apparatus for nse on an ordinary camera :—" Its chief advantage over the use of a Microscope with the camera being that the ordinary



use of the Microscope is not interfered with, so that the objects can be examined in the latter, and selected parts immediately photographed. A brass dise or adapter A, shown in section in the illustration, screws into a flange on the camera, and has a screw-threaded aperture to receive ordinary object-glasses. The disc earries a triangular rod B, abont  $3\frac{1}{2}$  in. in length, on which a square stage C slides, or can be clamped in any position by a screw D. The stage has a

central opening for light and springs for holding a slide. The back of the camera is movable to a distance of 20 in. by a screw; but an ordinary quarter-plate camera, with a rack and pinion, serves almost as well, the magnification then obtainable being less, owing to the shortness of

\* Engl. Mechanic, lv. (1892) p. 61.

such cameras. Focusing is effected by clamping the stage so that the object is approximately in focus, and then moving the back of the camera as found necessary. This apparatus, which I designed and had made early in 1881, has been found to work very well with low powers, the illumination being effected by a paraffin lamp with one wick, the flame of which is focused on the object by stand condensers, with a blue cell interposed."

## (5) Microscopical Optics and Manipulation.

Determination of the Focal Length of Optical Systems.\*—Prof. Abbe describes a very convenient apparatus for determining focal lengths. Most of the methods for this purpose depend on the determination of the position or displacement of the images formed by the system to bo tested. Such processes, besides being very difficult of application to systems of great focal length, are subject to considerable uncertainty. The method here suggested depends on the determination of the ratios of the apparent magnitudes of objects which are first observed directly and then by the system under examination. If this ratio for two objects at a distance from each other  $\Delta$  is  $\rho_1$  and  $\rho_2$ , the focal length  $f = \Delta (\rho_2 - \rho_1)$ . To obtain great exactness a Microscope with Abbc diaphragm is used, in which only pencils with axes parallel to the axis of the Microscope are effective in the production of the image. The complete apparatus consists of a Microscope, with the adjustable stage of which two glass scales at a determined distance apart are rigidly connected. The system to be tested is mounted between the Microscope and scales. In passing from one scale to the other, separated from it by a distance of 50 mm., the objective is changed in order to facilitate the adjustment.

Microscopic Image of Transparent Bodies. †-Prof. Abbo explains the peculiarity of the phenomena which take place in the microscopic representation of bodies illuminated by transmitted light. In general, for each point of the object there is a corresponding point in the image. For bodics illuminated by transmitted light this is no longer the case. The rays from tho source of light will on emergence from the object convert each point of its surface into a luminous point from which pencils are emitted in all directions. If, in the formation of the image, all of these pencils are made use of by the instrument, the image will then correspond to the object. This will happen when the angular aperture of the objective is sufficiently large to include all parts of the diffraction spectra formed on the boundary of the object. But where this is not the case different images may be obtained from the same object by blocking out certain of the diffraction spectra forming the image. It is also possible to obtain similar images from two different objects, if only those parts of the pencils which are common to both objects are made effective in the formation of the images, while the rest are blocked out.

Dr. Czapski demonstrated the truth of these remarks by some experiments on gratings made with a projection apparatus. By blocking out those spectra which resulted from the effect of every second line, the

\* Zeitschr. f. Instrumentenk., xi. (1891) p. 446, † Tom. cit., p. 447.

image of a grating became that of one with double the interval; and the images of two gratings of which one had double the interval of the other could in this way be made precisely the same. The image of two gratings at right angles to each other could, by making use only of the spectra lying in a diagonal direction, be converted into that of a grating with direction of the striæ along the diagonal of the gratings.

Investigation of the Action of Nicol's Polarizing Eye-piece.\*— Dr. Sang in this paper, which is now printed for the first time, although read on February 20th, 1837, explains the mode of action of the ordinary Nicol's prism, showing how the ordinary ray is removed by total reflection at the surface of the Canada balsam. He determines by mathematical calculation the correct inclination of the ends of the prism, and finds that the obliquity of the faces in the natural rhomb must be diminished  $4^{\circ}$  instead of increased as suggested by Nicol, the inventor, in his first description of the prism. A long and complex piece of analysis then follows to determine the best form of rhomb in order that the extra of field may be as large as possible. The author concludes with the suggestion that a polarizer might be constructed of two prisms of highly refractive glass, separated by a thin plate of Iceland spar cut at right angles to the axis.

In a note on the above paper, read November 23rd, 1891, Prof. Tait states how, at the urgent request of the late Dr. Sang, he brought before the Council of the Royal Society of Edinburgh the question of its publication. Whatever judgment may be passed on the rest of the work, the paper contains a very important suggestion in the proposal to construct a polarizer of two glass prisms separated by a thin layer only of Iceland spar. With regard to Dr. Sang's claim to priority in the explanation of the action of the prism, it is certain that many very similar attempts at explanation have been published since 1837, and the inventor himself supposed the action to be due to increase in the divergency of the two rays.

In place of the complicated piece of analysis in Dr. Sang's investigation of the limits within which the prism acts, Prof. Tait gives a much simpler demonstration. The employment of glass prisms separated by a thin layer of Iceland spar has been recently suggested by M. E. Bertrand.<sup>†</sup>

SCHELLBACK, H.—Der Weg eines Lichtstrahls durch eine Linse. (The Path of a Ray of Light through a Leus.)

Zeitschr. Phys. u. Chem. Unterr., IV. (1891). Centralztg. f. Opt. u. Mech., XII. (1891) p. 97.

#### (6) Miscellaneous.

Dr. Van Heurck on the Microscope ‡—This book is intended to be a comprehensive treatise on the Microscope as constructed and used in its present form. In its treatment of the elementary principles of

- \* Proc. Roy. Soc. Edinburgh, xviii. (1891) pp. 323-40.
- † Comptes Rendus, xcix. (1884) p. 538.

† 'Le Microscope, sa construction, son maniement; la Technique microscopique en général; la Photomicrographie; le Passé et l'Avenir du Microscope, par le Dr. Henri van Heurck,' 4th ed., Anvers, 1891, 8vo, 316 pp., text illust. optics there is nothing in any sense new, and there is neither in this part nor the subsequent pages of the book any effort made to give any mathematical expression to the complex modern theories of microscopic This will not diminish the value of the book to the less vision. ardent student; but it will leave a proportion of those who desire to understand fully the principles of modern theory and practice con-cerning the Microscope still unsatisfied; of course the one might have been accomplished without detriment to the other. "La théorie de la vision microscopique de M. le Prof. E. Abbe" is given elearly, but scarcely as exhaustively as we are inclined to believe its importance at this time demands. At length the theory of Abbe has received the careful consideration its value demands; but this has also brought with it a criticism which cannot be ignored, and the value of which will only be seen after an equally careful consideration. Like all similar criticiism it will only lead to what all carnestly desire, a more radieal analysis, and a firmer establishment and more useful application of what will remain unshaken. But it is all the more needful that those concerned in the controversy should have before them, in as elear and exhaustive a form as possible, the details of the great modern theory of microscopic vision.

In dealing with the "general consequences" of the theory there is certainly a fearlessness and an assumption of the inevitable in regard to these "consequences," which practically conclude that they may in their smallest details be placed side by side with the demonstrated laws of light. We much prefer an open mind, and although we believe that the Abbe theory in the main must remain unshaken, Dr. Abbe has himself shown that there are points in detail which have needed modification.

In the use of the more powerful object-glasses Dr. Van Heurck has shown himself singularly competent, especially in regard to photomicrography; hence we are somewhat disappointed to find so limited a range of "test-objects" considered, especially as those he directs us to employ have been for so long in use. What we now need is objects that will specifically differentiate the qualities of the apochromatic objectives from their achromatic predecessors, especially when it is considered that for lined (or "dotted") objects the table so long and usefully printed at the back of this Journal gives the theoretical power of any lens.

We note that the pygidium of the flea is given with much commendation as a "test." No doubt it has its value for this purpose; but with the recent (apoehromatic) objectives there is more discoverable than was taken as evidence of "test structure" a few years ago. The minute hair-like bodies covering the surface, and especially their mode of insertion, are among these.

Of course we have a slight reference only to the new object glass by Zeiss of N.A.  $1 \cdot 60$ , and Dr. Van Heurck's remarkable photomicrographs with it are reproduced. In regard to these it must be remarked that they can hardly be counted satisfactory, taken as they were with a condenser whose aberrations are of the most emphasized nature, and with no attempt at their correction.

The disqualifications of this remarkable objective are not referred

to or illustrated, but there is a final chapter, in the form of a translation of a paper by Dr. S. Czapski, which gives a suggestion for the possible enlargement of the practical N.A. of homogeneous lenses, which gives us the possibility of advancing to  $2 \cdot 0$  or beyond it without the employment of the dense flint and high refractive media needed by the lens we have referred to. This communication has already appeared in English in this Journal, and indicates clearly the value of *true* monochromatic light which may increase a N.A. of  $1 \cdot 40$  to  $1 \cdot 75$ .

There is a considerable chapter on "Photomicrographie." This, as a matter of course, is excellent, but is not exhaustive nor as specially critical in regard to instruments and methods as might bo desired.

As must inevitably be the case in all general treatises on the Microscope, the chapter on the preparation and mounting of objects is suggestive, and, so far as it goes, useful, but inefficient. But the student may well be content, for many handbooks are now obtainable wholly devoted to the subject.

In regard to the important question of what a Microscope should be, what are its points of excellence, what is indispensable, and consequently what should be its general form and detailed construction, we do not obtain very definite conclusions. A large array of Microscopes are presented in an illustrated form, but they are mostly described, without being critically compared. We have, however, a figure of an instrument prepared by Messrs. Watson for Dr. Van Heurck, described and illus-We presume this will be more or less the expression of what trated. the author desires in a Microscope. It is no doubt fitted with what are intended to be the latest improvements; but, to begin with, it would be held by most experienced workers, especially photomicrographists, that the stand is mest defective from its form, involving lateral instability, and securing such stability as it possesses by weight rather than by construction. But this is secondary to the want of perfection in its fineadjustment and the mode of centering in its substage, to say nothing of the awkward position of the milled head employed, apparently, in giving a fine-adjustment to the substage, and the great thickness of the stage itself.

The book is well printed, and fairly illustrated; it gives a brief history of the instrument it describes, and instructs the reader how to use it. It will be of service to many, and throughout gives valuable hints, as well as lucid instruction, to those who seek to understand how to use to the best advantage one of the most delightful instruments employed in the study of nature.

Text-book of the Microscope.\*—This is one of the most practical books yet presented to the laboratory student. The aim is to enable him to understand his instrument in at least its broader principles, and thus to apply it intelligently to the study of histology. It cannot be doubted that there is, chiefly perhaps because of the great variety of subjects requiring to be dealt with in the medical curriculum, a deficiency of knowledge amongst students as to the principles and the optical laws involved in the construction and use of the Microscope. Prof. Gage

\* 'The Microscope and Histology, for the use of laboratory students in the anatomical department of Cornell University, by Simon Henry Gage. Part I. The Microscope and Microscopical Methods.' 3rd ed. Ithaca, N.Y., 1891, 8vo, 96 pp. interleaved, 5 pls., text illust. evidently feels this, and remembering the little time at the disposal of the student, has sought to condense and put in the most concise and practical form what it is well for him to know about his instrument in theory and practice. We cannot say that a student can consider his knowledge as either wholly satisfactory, or in any sense exhaustive, when derived from this book alone; but on the points in difficulty he can readily consult more exhaustive treatises. The illustrations are crude, but they are only intended to be diagrams, and are in tho main made to convey the points specially emphasized.

On mounting there are some good practical hints, and there is a very useful bibliography, with an index completing the volume. We conclude that this book has a *raison d'étre*, and will, especially in America, command a good audience.

Behrens' Tables for Microscopists.\*—The appearance of this second edition of Behrens' useful tables will be welcomed by microscopists. The new edition forms really a new work, for few only of the tables of the original remain unchanged. In tho preparation of this greatly enlarged edition, Dr. W. Behrens has had the assistance of several eminent authorities. The 76 pages of the first edition have been extended to 205, while the number of separate tables has been increased from 54 to 76. The arrangement of the tables remains very much the same as in the first edition. The most important addition consists in two long tables devoted to microchemical reactions, for botanical and mineralogieal investigations.

Nature of Solutions and the Use of the Microscope.<sup>†</sup>—In a discussion on solution and pseudosolution, Mr. H. Jaekson remarked that he had found in the case of lead hydrate that the Microscope revealed moving particles with an average diameter of 1/35,000 in. Some, in the case of silver nitroprusside, were less than 1/100,000 in. It is quite clear that it is impossible to call a liquid homogeneous because the Microscope fails to show structure. All that can be said is that the particles in water, if not visible with lenses of the greatest possible angle for water, are probably not much greater than 1/180,000 in. in diameter.

The late Mr. W. W. Reeves.—The Fellows will hear with regret of the death of one of the most constant attendants at our meetings, and one well known, especially to the older Fellows, in consequence of his having been Assistant Secretary to the Society from 1868 to 1883. Ho has served on the Conneil since 1890. Our deceased friend died on the 18th of May last.

 DUVAL, A.-LE technique microscopique et histologique. (Microscopical and Histological Technique.)
 Paris (Baillière) 1891, 16mo, 43 figs.
 S EHIEFFERDECKER, P., U. A. KOSSEL-Gewebelehre mit besonderer Berücksichtigung des menschlichen Körpers. [Bd. II. von W. BEHRENS, A. KOSSEL U. P. SchleffErdecker, Die Gewebe des menschlichen Körpers und ihre mikroskopische Untersuchung.] (Histology, with special reference to Man (Vol. II. The Tissues of the Human Body, and their examination by the Microscope.)

1. Abtheil, Braunschweig, 1891, 8vo, 414 pp., 214 figs.

\* Behrens, W., 'Tabellen zum Gebrauch bei mikroskopischen Arbeiten. Zweite neu bearbeitete Auflage,' Braunschweig, 1892, 8vo, 205 pp.

† Proc. Chem. Soc. London, No. 104 (1891) pp. 178-9.

#### B. Technique.\*

#### (1) Collecting Objects, including Culture Processes.

Mode of keeping Fresh-water Animals alive.<sup>†</sup>-Dr. J. Dewitz recommends the spreading of a piece of canvas or small dampened towel over the bottom and side of a plate on which the fresh-water animals are placed. They must then be covered with another wet towel and put in a cold room. The towels must be washed and wrung out every fourth day, and any dead specimens removed.

Antibacterial Value of Aristol. +-- In order to estimate the antibacterial value of aristol, Dr. Heller inoculated agar plates with Staphylccoccus aureus, putrefactive bacteria, and anthrax. Some of the plates were covered with iodoform, and others with aristol. The capsules were then incubated at 37°.5 for 2-3 days in the absence of light, sufficient moisture being supplied by water placed in other capsules. No further development was found on the plates strewn with iodoform, but on those covered with aristol there was a luxuriant growth, except where the layer happened to be very thick, a result probably due to the mechanical exclusion of air. Hence it would seem that the antibacterial value of aristol is small, and, at any rate, not to be compared to that of iodoform.

Effect of Centrifuging on Bacterial Suspension, with special reference to the Dissemination of Bacteria in Milk.§-After having ascertained by experimenting with anthrax that an hour's centrifuging at the rate of 2000-4000 turns a minute was not detrimental to the vitality or the virulence of these organisms, Herr Scheurlen turned his attention to the behaviour of bacterial pure cultivations in suspension.

The results of centrifuging were found to depend on the mobility or immobility of the bacteria, the latter tending to be thrown out and to form a sediment, while some of the former, e.g. Cholera vibrio and Proteus mirabilis, remained suspended.

The author then examined the behaviour of the bacteria of milk when similarly treated. After centrifuging, the milk-scum, when tested by means of plate cultivations, showed, as was to be expected, a large number of colonies, while the number in the cream was also very great, and might even exceed that of the scum.

The author infers from the experiments that milk cannot be freed from its bacteria by centrifuging, for out of 2050 millions of germs in the whole volume of milk, only 18 millions were removed in the scum. About three-fourths of the number are transferred by the centrifuging to the cream, the remainder being in the buttermilk.

Most pathogenic microbes, such as anthrax, typhoid, and cholera, cling to the cream like the milk-bacteria, but tubercle bacilli were for

\* This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes; (4) Staining and Injecting: (5) Mounting, including slides, preservative fluids, &c.;
(6) Miscellaneous. + Zool. Anzeig., xv. (1892) pp. 105-6.
‡ Arch. f. Derm. u. Syphilis, 1891, p. 840. Sce Centralbl. f. Bakteriol. u. Para-

sitenk., xi. (1892) p. 351. § Arbeit. a. d. Kaiser. Gesundheits-amte, vii. (1891) See Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 53-4.

the most part separated out, only a few remaining in the milk and cream.

Collecting Samples of Water for Bacteriological Analysis.\*—Dr. W. Johnston describes in detail his method of obtaining samples of water from any depth, free from contamination and with great ease. The collecting apparatus, which is a modification of that invented by Prof. Ellis, is very ingenious. It consists of a framework holding a sterilized glass bottle, and so weighted that it can be lowered to the required depth. By pulling a string attached to the stopper the latter can be raised sufficiently to allow the water to enter. When the string is released, the stopper is instantly replaced by the action of a spring.

For further details of this simple and ingenious apparatus the original must be consulted.

Method of obtaining Pure Cultivations of Tubercle Bacilli from the Sputum.†-Dr. E. Pastor first ascertains if the sputum contains a good quantity of bacilli and relatively few foreign organisms; the patient is then made to repeatedly wash his mouth and the back of his throat out with sterilized water, and then to expectorate into a sterilized vessel. The sputum thus obtained is emulsified by shaking it up with sterile water, and any coarse particles filtered off with fine gauze. A few drops of the filtrate are then mixed with fluid 10 pcr cent. gelatin, care being taken that the medium is not rendered too cloudy. The still fluid gelatin is then poured out into plates, and these, just covered with a bell-jar, are left at the room temperature. In the course of three to four days colonies of bacteria spring up. By means of a lens parts of the gelatin which remain quite clear are then sought out. These are then carefully excised with a sterilized knife, and inoculated on obliquely-set bloodserum. Of ten blood-serum tubes inoculated in this way the author obtained always one, and in some several, pure cultivations of tubercle In the rest of the tubes impurities appeared owing to the bacilli. development of germs, which at 37°.5 and on blood-serum overpowered the tubercle bacilli.

From the fluid contents of phthisical cavities, of course, better results are obtained, as this material contains not only more bacilli but less impurities.

## (2) Preparing Objects.

New Method of Preparing Sections of Teeth and Bone, to demonstrate the hard and soft tissues in combination. $\ddagger$ —Mr. A. Hopewell Smith writes:—" Immerse a newly extracted tooth in Müller's fluid for three to four weeks, and remove to spir. vini rect. for ten to twenty days. Alcohol (84 per cent.) may be used instead of Müller's fluid. Remove, wash in water, and seal up apical foramen with collodion. Place tooth in 15 ccm. of following solution:—HCl, 12 parts; HNO<sub>3</sub>, 30 parts; aq. dest., 108 parts. Take 12 ccm. of 10 per cent. solution of HCl, and at end of fifteen hours add 1.5 ccm. of HNO<sub>3</sub>, from commencement

1892.

2 н

<sup>\*</sup> Canadian Record of Science, 1892, pp. 19-28 (1 pl. and 5 figs.).

<sup>†</sup> Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 233-4.

<sup>‡</sup> Trans. Odont. Soc. Gt. Britain, xxiv. (1891) p. 20.

of immersion in acid solution. Remove tooth (molar) at end of seventyfive to eighty hours, and wash in solution of lithium carb. (5 grm. to an ounce) for half an hour. Wash thoroughly with distilled water. Divide tooth by razor into several pieces, and wash again in water. Place each piece in gum mucilage (B. P.). Leave in mucilage twelve to fifteen hours. Transfer to stage of freezing microtome, cut, wash sections, and stain with orange-rubine, or gold chloride, or boraxcarmine, or Weigert's solutions. Dehydrate in absolute alcohol three minutes, clear in cedar oil one and a half minutes, and mount in Canada balsam."

Investigation of Brain of Marmoset Monkey.\*-Dr. C. E. Beevor, who has studied the fibres of the cingulum and the corpus callosum and fornix in the Marmosets, put the brains direct into a 3 per cent. solution of bichromate of potassium, where they were hardened from two to four months, and in one case for twelve. After hardening the brain was imbedded in celloidin and cut into sections by Schanze's microtome. Weigert's hæmatoxylin method, or Pal's modification thereof was used for staining. The sections were dehydrated by absolute alcohol, clarified by oil of cloves or origanon oil, and mounted in Canada balsam. The advantage of origanon oil over oil of cloves is that it does not dissolve out the celloidin, which is thus able to hold together the fincr parts of the section, and prevent it from falling to pieces in the process of clarifying. The objection to it is that the sections do not remain perfectly flat. To obviate this some of the sections were dehydrated on the slide, the absolute alcohol was run off without disturbing the sections, which were then clarified by adding origanon oil. When this was completed the oil was run off, and oil of cloves was carefully added. This was run off after dissolving the celloidin.

Some of the sections were cut after imbedding in paraffin. The brain was hardened in methylated alcohol, put for two weeks in a 3 per cent. solution of bichromate of potash, washed in water and in methylated alcohol, and put direct into Weigert's hæmatoxylin for three or four days.

Preparation of Eggs of American Alligator.<sup>†</sup>—Mr. S. F. Clarke carefully removed the shell and its membrane from nearly half one side of the egg; the contents were then poured out on to the left hand, and the thick white at one end cut off with the scissors. The egg is then replaced in the shell with the germinal pole, still covered with white, uppermost. The white is next cut off as completely as possible from this end. The egg is now set on end, and a sharp-edged lifter is used to press out on either side incisions made with sharp-edged scissors. By this means the portion of the blastoderm which contains the embryo, and measures from 12 to 15 mm., is separated from the rest. The embryo is treated with Kleinenberg's picric for sections, or chromic acid for surface study.

Preservation of Tadpoles.<sup>1</sup>—Mr. G. A. Boulenger recommends the collector of tadpoles to provide himself with small test-tubes half-full

- \* Philosoph. Trans., 182 B (1892) pp. 137-8.
- + Journal of Morphology, vi. (1891) pp. 202-3.
- ‡ Proc. Zool. Soc. London, 1891 (1892) p. 599.

of weak spirit; the tadpoles, when taken out of the fishing-net, should be dipped head foremost in the tube. On reaching home the liquor should be at once changed to spirit 40 over proof, and this must be changed daily till it ceases to be strongly coloured. This mode of treatment preserves the natural shape of the tadpole, and the delicate caudal crests do not shrivel. Tadpoles should never be allowed to remain out of the fluid, as they shrink very rapidly. Chromic acid is not to be recommended, as it renders the specimens too brittle for ordinary study.

Preservation of Invertebrates in a State of Extension.\*—Herr T. Tullberg was guided in his researches by the *a priori* consideration that, as sea-water contains several salts in definite proportions, it was probable that marine animals would not contract if one was to increase the proportion of one of the salts of the water; for the animal is already accustomed to these substances, and, on the other hand, it might have a toxic effect. Experimenting with *Actiniæ* (and especially with *Actinoloba dianthus*) he found that chloride of sodium had no effect, but with sulphate or chloride of magnesium the Actinian expanded its tentacles, and, after a certain time, did not contract at all when its tentacles were pinched.

It is first necessary to get an Actinian into a state of expansion; this may be done by leaving it for some time, even twenty-four hours. in a vessel of sea-water. The quantity of water must be measured so as to know the percentage of salt which is to be added. These precautions having been taken, a 33 per cent. solution of chloride of magnesium is added until the vessel contains 1 per cent. of the salt; thus for one litre of sea-water 30 ccm. of the solution must be added. The addition should be made slowly, or even intermittently; but it must be effected within half an hour. Thirty minutes later the animal will be found to be anæsthetized. As a matter of fact only the exterior of the animal will have lost its sensibility. The animal must now be killed; if the animal be inundated with alcohol, concentrated chromic acid, or Perenyi's fluid the result may be satisfactory for anatomical or histological purposes, but the specimens will not be fit for exhibition in a If the fluids are added slowly, better results will be obtained. museum. With chromic acid the following procedure may be adopted :-- a tube with a funnel is plunged as far as the bottom of the vessel in which the animal lies, and a ·1 per cent. solution of chromic acid is slowly and intermittently added. If the animal begins to contract during the operation, it must be stopped for a short time. The addition of acid must go on until the chromic acid is in the proportion of .3 to .5 per cent. of the total liquid. If the animal does not contract when this solution is added, the dose of chromic acid must be increased till the proportion is ·5 per cent.

The results of this method are very satisfactory, save that there is a decrease in the volume of the animal. Sections of the tentacles showed that the cells were not attacked by the substances employed. Various animals have been experimented on. The author has applied this process to terrestrial and fresh-water Invertebrates, and finds that chloride

\* Arch. Zool. Expér. et Gén., x, (1892) pp. xi-xiv.

2 н 2

of magnesium completely anæsthetizes them; it is well to use rather stronger solutions (about '4 per cent.) for them.

Observation of Blood of Astacus.\*-Mr. W. B. Hardy obtained the blood of the Crayfish by aid of a glass pipette drawn out to a fine point and pushed through the soft skin which joins the dorsal portion of the cephalothoracic shield to the tergum of the first abdominal appendage. The pipette is fitted with a strong india-rubber bag, by the aid of which fluid may be sucked up into the tube. The skin is stretched by pushing down the abdomen of the Crayfish, and the point of the pipette is inserted from the side and horizontally so as to avoid injuring the heart and superior abdominal artery. A drop or two of blood being sucked up, the abdomen is quickly bent upwards so as to mechanically close the tiny aperture. A blood-clot will soon form, and the Crayfish may be replaced in the tank without any further loss of blood.

Permanent preparations may be made by inverting a drop of blood over a 2 per cent. solution of osmic acid; after 15 minutes the blood can be stained and mounted in glycerin or balsam. A reagent of the very greatest value in the study of the corpuscles is iodine.

Study of Cutaneous Glands of Crustacea.<sup>†</sup>-M. M. Ide fixed small marine species, such as Vibila or Phronima, by plunging them entire into Kleinenberg's solution, picrosulphuric acid, or 70 per cent. alcohol. With larger species, such as Asellus or Oniscus, the head was cut off and plunged in water saturated with corrosive sublimate or Gilson's solution (acid sublimate). For the glands of the urostyle of Oniscus, which are very difficult to reach, the ends of the urostyles must be cut off, and Gilson's solution injected into the body of the animal till it escapes by the cut extremities; the caudal portion must then be cut off with scissors and left for ten minutes in a bath of the fixing solution. Sections should be coloured on the slide either with alum picrocarmine alone or by it, followed with picric acid or watery blue carmine.

Study of Compound Eyes of Annelids.<sup>‡</sup>-Mr. E. A. Andrews macerated the eyes of Annelids in Bela Haller's liquid, in potassium bichromate, and in sea-water containing a small amount of sulphuric acid; the last method was found very useful. The staining reagents used were Mayer's acid carmine, Czokor's cochineal, and methyl-green. Sections were stained with Kleinenberg's hæmatoxylin, and Grenacher's fluid was used for dcpigmentation.

Examination of Ciliated Organs of Hirudinea.§-Prof. H. Bolsius has, in his general study of the segmental organ, chiefly employed the method of serial sections; these, it is to be observed, were not made on extirpated organs, but on entire individuals. This is the most advantageous method, as the organs are too delicate for ordinary dissection. In tcased preparations the best staining agent was found to be methyl-green.

Preparation of Echinoderms. - Mr. G. W. Field recommends the following method of preparing large starfishes, such as Oreaster. Kill

<sup>\*</sup> Journal of Physiology, xiii. (1892) pp. 165-6.

<sup>†</sup> La Cellule, vii. (1891) pp. 352-3.
‡ Journal of Morphology, v. (1891) pp. 272-7.
§ La Cellule, vii. (1891) p. 296.

John Hopkins Univ. Circ., xi. (1892) p. 84.
by immersion in fresh water, at  $35^{\circ}-40^{\circ}$  C., for an hour or two. Remove as much as possible of the soft parts. Immerse for any convenient time in alcohol in which corrosive sublimate is dissolved. Dry very quickly in the hot sun. After thorough drying, the normal colours can be reproduced by means of water colours with very good and permanent results.

Satisfactory specimens of *Diadema setosum* are particularly difficult to procure, on account of the delicacy and fragility of the spines. Thrust a stout sharpened wire through the anal membrane as the creature lies in his natural position in the water; when detached from the bottom, push the wire through and make a loop. Suspend the animal for 15 minutes in warm fresh water, and in 1 per cent. chromic acid solution in a large vessel; wash in several changes of fresh water after using chromic acid; place for an hour or two in weak alcohol, then for a day or two in 10 per cent. solution of corrosive sublimate in strong alcohol. Dry rapidly in the sun, suspended by the wire. If the drying be quickly done, the spines will retain nearly their normal position. This process, with modifications, can be applied to other Echinoids.

Alcohol specimens should be killed by immersion for a short time in 0.1 per cent. chromic acid solution, or in warm fresh water; then place in weak spirit, and finally in 80 per cent. alcohol. For histological purposes Flemming's chrom-osm-acetic solution gives good results. After hardening recalcify in 70 per cent. alcohol to which a few drops of hydrochloric or picronitric acid has been added.

Methods of Examining Zoantheæ.\*—Prof. A. C. Haddon and Miss A. M. Shackleton examined specimens of Zoantheæ which had been preserved in alcohol. When a sufficient quantity of strong spirit is used this answers vcry well. They stained the objects alive in boraxcarmine, imbedded them in paraffin, and cut them with a rocking microtome. The unincrusted and some of the incrusted genera are very easy to cut. As a rule, the incrustations from coral seas are calcareous, and admit of being readily dissolved away with nitric acid.

Mode of obtaining Sections of Ovules.<sup>†</sup>-Herr J. W. Moll recommends the following method of making sections of ovules :- The ovules (Fritillaria imperialis) are placed for twenty-four hours in Flemming's solution; after washing with water they are transferred to alcohol of about 95 per cent., and are then dissected in alcohol under the lens. The parietal protoplasm of the embryo-sac is removed and placed in a drop of celloidin and solidified, and the slide is then plunged in alcohol of 95 per cent. The thin plate of celloidin containing the material for the sections is then detached from the slide and placed in alcohol of 95 per cent. coloured by gentian-violet for an hour, next in a mixture of 6 per cent. of oil of marjoram and 1 per cent of alcohol (95 per cent.), and then in pure oil of marjoram until it becomes transparent. The plate of celloidin is imbedded in paraffin and cut into thin sections, which are mounted in Canada balsam or dammar-resin after staining with gentian-violet. The object is to obtain the nuclei in process of division.

<sup>\*</sup> Scient. Trans. Roy. Dublin Soc., iv. (1891) p. 611.

<sup>+</sup> Bot. Jaarb., xx. (1890) p. 325. See Bonnier's Rev. Gén. de Bot., iv. (1892) p. 81.

#### (3) Cutting, including Imbedding and Microtomes.

A new Method of Using Celloidin for Serial Section Cutting.\*— The following method is recommended by Mr. H. C. Bumpus as having several features which make it preferable to the ordinary methods of section-cutting :—It allows a perfect orientation; the entire object is visible during the process of cutting; yolk-bearing eggs offer no serious difficulty; sections of large area and of unusual thinness are easily secured; crimping and curling during the process of clearing are avoided and the sections may be readily arranged in series.

The object is first stained *in toto*, dehydrated, infiltrated with thin, medium, and thick celloidin or collodion (Squibb's flexible collodion rendered thick by evaporation is excellent) and finally placed in a paper tray filled with the thick collodion. In a few moments a film will form over the exposed surface of the collodion, when the paper tray with its contents is thrown into a jar of strong chloroform, in which, after a few hours, the collodion becomes quite hard.

The tray is now taken from the chloroform, and, after the paper has been removed from the hardened block, the collodion with its enclosed object is placed in a vial of white oil of thyme, or some other similar oil. If the block of collodion is not large, in a few hours it will become as clear as glass, the stained object appearing as if suspended in a transparent fluid.

For the process of orientation, the block of collodion may now be taken from the oil, placed in a watch-crystal, and, after covering with the oil of thyme, examined with a lens, or, if more desirable, with a compound Microscope. The side of the block that is to be attached to the object-holder of the microtome is now selected, wiped dry of the oil, and immersed for a moment in ether, and then smeared with thick collodion. The object-holder, a block of wood rather than cork, is smeared in the same way, and the two collodionized surfaces are brought together. The holder and collodion block are now immersed for a few minutes in chloroform, or long enough for them to become firmly united.

The preparation is now screwed between the jaws of the objectcarrier of the microtome and covered, by means of a camel's-hair brush, with oil of thyme. The microtome knife is flooded with the same oil. This cil, which takes the place of alcohol usually used, has the advantage, because of its lubricating property, of not only permitting thin sections to be cut, but its slow evaporation allows one to leave work at any time for minutes or even hours without the object being injured.

After a fcw sections have been cut from the block of collodion, the relative position of the plane of the knife to the axis of the object can be definitely established. There is no difficulty in orienting small Arthropod embryos by simply examining the object and plane of cutting at this time with a compound Microscope; the segments, appendages, and even nuclei are as clearly shown as if mounted in balsam. The object, satisfactorily oriented, is now cut and the sections at once transferred to the slides, covered with balsam and mounted, or, if they are not immediately needed, they may be kept indefinitely in a vial of the oil.

\* Amer. Nat., vevi. (1892) pp. 80-1.

438

If the sections are to be arranged in "series," they are simply placed upon a slide one after the other, care being taken not to flood the slide with oil but to keep it quite dry. After the sections are arranged, the slide is tilted up to allow the excess of oil to drain away, fifteen minutes generally being sufficient. Balsam is now placed on the sections and a warm cover is allowed to gently fall over the series, no section of which ought to leave its place.

The above method is especially useful in the preparation of larger yolk-bearing eggs.

### (4) Staining and Injecting.

New Method for staining Central Nervous System.\*—The pieces of nervous tissue are, says M. Lichen, to be placed for five weeks in a mixture of equal parts of 1 per cent. chloride of gold and 1 per cent. corrosive sublimate. The pieces are then sectioned in dilute lugol solution (1/4).

The medullated or non-medullated fibres, the nerve-cells, and the neuroglia-cells are coloured blue. In the ganglion-cells nucleus and nucleolus were clearly differentiated.

Methods of staining the Axis-cylinder in Sections of Spinal Cord.<sup>†</sup> —For staining spinal cord Herr Schmaus adopts the following modification of Gierke's method :—1 grm. of carminate of soda, 1/2 grm. of nitrate of uranium, and 100 grm. of water are heated for half an hour and filtered when cold. The sections are placed for 15 to 20 minutes in the staining fluid and then washed with water.

The tissue must have been previously hardened in Müller's fluid. By this method the axis-cylinders are perfectly stained while the celloidin is unaffected.

In another method employed by Kronthal, carmine is used with great success. A saturated solution of carmine is made in ammonia, and the solution, merely covered with a piece of tracing paper, is allowed to stand for a week. The supernatant fluid is then decanted to a wellstoppered bottle and left for four weeks.

In a mixture of 10 drops of this fluid and 100 ccm. of distilled water the sections are placed for 24 hours, after which they are washed with water for 24 hours. The older the solution is the greater its staining power, due to the formation of the carbonate of carmine, becomes. It does not stain the celloidin.

A third method recommended by Schmaus is to stain the axiscylinders with English blue-black. The solution consists of 1/4 per cent. blue-black to 50 per cent. spirit with a little picric acid added. After an immersion of one hour the sections are washed and mounted.

Examination of Nerve-centres by Iodide of Palladium Process.<sup>‡</sup>— Prof. G. Paladino states that his process of studying the central nervous system consists in impregnation by chloride of palladium in a 1 per 1000 solution, and formation of iodide of palladium by the reaction of iodide

† Münchener Med. Wochenschr., 1891, No. 8. See Bull. Soc. Belge de Microscopie, xviii. (1891) pp. 11-12.

‡ Journ. de Micrographie, vi. (1892) pp. 77-8

<sup>\*</sup> Neurol. Centralbl., 1891, No. 3. See Bull. Soc. Belge de Microscopie, xliii. (1891) p. 13.

of potassium in a 4 per 1000 solution. Immersion in chloride of palladium may be continued for two or three days or even weeks without danger, and even with advantage, if the solution be renewed; but immersion in iodide of potassium must not be for more than one or two hours. Those who have used these reagents speak highly of them.

Useful Modification of Gram's Method.\* — Dr. Eng. Bothin says that many of the well-known inconveniences incidental to Gram's method may be obviated, and the staining of the bacteria much improved by washing the preparation, section or cover-glass, in anilin-oil-water after it is removed from the gentian-violet solution, and before it is immersed in the iodine solution.

Genevan Reagent.<sup>†</sup>—Under this name (réactif genevois) Prof. R. Chodat recommends a double staining reagent which he finds useful in the differentiation of vegetable tissues. It consists of a slightly alcoholic and ammoniacal solution of congo-red (2 per cent.) and chrysoidin (2 per mille). The section is first decolorized by eau de Javelle, and then immersed in this reagent for a few seconds, when a beautiful double or triple staining is obtained. The cellulose membranes are coloured rose, while the lignified or cutinized cells take a yellow tint varying according to the degree of hardening of the membranes.

#### (5) Mounting, including Slides, Preservative Fluids, &c.

Fixation and Preservation of Compressed Objects.  $\ddagger$  — Mr. N. A. Cobb suggests the following method for fixing, staining, and mounting compressed objects. The object—e. g. a dipterous larva or a Nematode—is compressed between two small cover-glasses of the same size; the amount of compression must be regulated by means of two hairs, or, better, two pieces of spun glass. The animal should be laid on one of the covers in a drop of water too small to entirely fill the space between the covers. When the other cover is laid on and the object is correctly compressed and arranged, the covers must be fixed in place. This is done by moving the two covers to the edge of the slide by means of a needle, and touching first one side of the pair, then the other side, with the wick of a wax taper or candle which has just been extinguished. The melted wax serves to cement the covers together, and they may be afterwards handled without much risk.

The covers, thus united, must be allowed to lie until all or nearly all the water between them has evaporated. To further treat the animal, take an elongated piece of quill or other similar elastic non-metallic substance, and make in it two cuts in such a way as to convert the quill into a compressing machine; into the compressorium so made insert the covers. To fix the object, take hold of the quill and place one edge of the covers in the fixing fluid, which will run in by capillary attraction. The author recommends that the whole apparatus be made so small as to be readily introduced into the object-box of his differentiator.

- \* Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 231-2.
- + Arch. Sci. Phys. et Nat., xxvi. (1891) p. 500.
- <sup>‡</sup> Proc. Linn, Soc. N.S.W., vi. (1891) pp. 143-6 (3 figs.).

#### (6) Miscellaneous.

Muencke's Centrifugal Machine for Bacteriological and Clinical Examination Purposes.\*—Dr. R. Muencke has devised a centrifugal machine (fig. 56) for bacteriological and clinical purposes. The machine, which, as the accompanying illustration shows, is worked by hand, is intended to avoid the waste of time incident to sedimentation of fluids



containing elements of very small specific gravity. By the aid of this apparatus oxalic acid crystals, red discs, albumen, and even microorganisms, can be demonstrated under the Microscope in a moment, and for the examination of the urine and of any exudation it is of especial service.

The machine, depicted 1/5 the natural size, merely consists of a plate which is caused to revolve by means of a rackwork and wheel. One turn of the wheel causes the plate to revolve 50 times. Fig. II. shows the wheel rotating, and fig. III. a glass tube which is used to contain the fluids.

\* Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 85-7 (3 figs.).

# JOURNAL

# OF THE

# ROYAL MICROSCOPICAL SOCIETY;

# CONTAINING ITS TRANSACTIONS AND PROCEEDINGS,

AND A SUMMARY OF CURRENT RESEARCHES RELATING TO

ZOOLOGY AND BOTANY

(principally Invertebrata and Cryptogamia),

MICROSCOPY, &c.

# Edited by

F. JEFFREY BELL, M.A.,

One of the Secretaries of the Society and Professor of Comparative Anatomy and Zoology in King's College;

WITH THE ASSISTANCE OF THE PUBLICATION COMMITTEE AND

A. W. BENNETT, M.A., B.Se., F.L.S., Lecturer on Botany at St. Thomas's Hospital,
B. G. HEBB, M.A., M.D. (Cantab.), AND

J. ARTHUR THOMSON, M.A., Lecturer on Zoology in the School of Medicine, Edinburgh,

FELLOWS OF THE SOCIETY.

### FOR THE YEAR

1892.

Part 2.



PUBLISHED FOR THE SOCIETY BY WILLIAMS & NORGATE,

# MICROSCOPY.

# a. Instruments, Accessories, &c.\*

#### (1) Stands.



Messrs. Baker's New Microscope.—Appended is a figure of Messrs. Baker's new Microscope, a description of which was given when it was exhibited to the Society.<sup>†</sup>

\* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous. † See this Journal, 1891, p. 867.

#### 542 SUMMARY OF CURRENT RESEARCHES RELATING TO

A New Construction for the Microscope.\*-Dr. Hugo Schreder, in reference to the paper by Dr. Lendl in Zeitschr. f. Wiss. Mikr., viii. (1891) p. 281, of which an abstract appeared in the last number of this Journal, has anticipated many microscopists in pointing out the fallacy involved in the conception that any real increase in the capacity of the Microscope is obtained by replacing the eye-piece of a Microscope by a second auxiliary Microscope. He also shows that the idea of such a construction has not even the merit of being new. A similar construction was originally described by Prof. Listing in Carl's Repertorium, v. (1869) pp. 1-5, and 134-40, and an extension of the same idea was made by Prof. Piotrowskiego, † who used Hartnack lenses partly with negative foci, and went even farther than Dr. Lendl with his (empty) magnifications.

Errors of this description were possible at a time when the diffraction theory was not generally known, but they are inexcusable now when the work of Prof. Abbe should be familiar to every microscopist. Such an arrangement as that proposed by Dr. Lendl only serves to magnify the structures in the Microscope image which have been altered by diffraction, and does not tend to give a clearer definition of any detail than can be obtained by the use of good eye-pieces. The capacity of the Microscope is not to be increased by any such simple method as this. For such a purpose processes, such as the increase of the numerical aperture, which aim at the diminution and prevention of the errors of diffraction are the only means which can give any profitable result.

An All-around Microscope.<sup>‡</sup>-Prof. S. A. Forbes writes :- "My personal studies are of a kind to require a Microscope which may be used (1) for the study of bacteria slides, (2) for the study of mounted slides of serial sections, (3) to search through and examine carefully collections of minute alcoholics in glass dishes, (4) to dissect animals under powers varying from twenty to two hundred diameters, and (5) to study pinned insects in all positions.

For the first purpose one must have a stand fitted to carry objectives of the highest power and the best illuminating apparatus; for the second, something in the nature of a mechanical stage is very desirable, but this must have a far wider sweep than the ordinary geared stage; for the third, one must be able to explore rapidly and with low power a large surface, moving back and forth along parallel lines as with a mechanical stage, but with much freer motion in all directions. The stage must also be without surface projections or attachments which would be in the way of a glass dish of considerable size.

The instrument must, further, stand erect, and yet must not be too high to work at sitting. It is a great advantage if both eyes may be used.

Fourth, for dissection hand-rests must be provided, and the Microscope must usually stand erect, and should be a binocular. Fifth. for entomological work a binocular is needed, with stage socket for insect forceps, and with a large central opening in the stage to allow

 \* Central-Ztg. f. Optik u. Mechanik., xiii. (1892) p. 98.
 \* O Mikreskopach I Teleskopach," in Osobne od bicie, z. xxxix. Tomu Boczu ‡ Amer. Mon. Mier. Journ., xiii. (1892) pp. 91-2. Tow. nauk. krak.

the ready turning of the object without interference of the pin or danger of injury to the specimen. As this large opening will admit light beside the condenser in bacterial work, it must be fitted with an adapter with a smaller opening. In this work, also, the rectangular movement of a mechanical stage is a great convenience for bringing insects readily into the field.

As I accomplish all these purposes perfectly by a single Microscope, it seems to me that this instrument must be adapted substantially to everything which the biologist is likely to want to do with a Microscope, and that a description of it may interest many situated similarly to myself.

My starting point is a Zeiss stand, No. 1, with oculars 1 to 5 (No. 3 being divided for the insertion of a micrometer), and objectives ranging from  $A_3$  to an apochromatic 1-12, with the corresponding eye-pieces. For ordinary binocular work I have a Zeiss binocular eye-piece, which has the advantage over any other binocular arrangement that it does not so increase the height of the instrument as to make it inconvenient to use it with low powers while sitting.

The special feature of the instrument is the stage, which is the simplest form of mechanical movement in two rectangular directions,



Fig. 58.

adapted to the square stage of my instrument as illustrated by the engraving accompanying (fig. 58). The stage-plate of the Microscope is altered only by a triangular groove along the whole length of the lateral margin, and by an enlargement of the central aperture. Into this a heavy diaphragm may be slipped with an opening of the usual size for the Abbe condenser.

# 544 SUMMARY OF CURRENT RESEARCHES RELATING TO

The apparatus for mechanical movement is essentially the ordinary mechanical stage, but working directly by hand instead of by rack and pinion, the especial advantage being the free long movement thus permitted. It is in two parts. A rectangular frame (a) showing front margin (g), as seen from behind, the lateral bar of which is bevelled to fit into the triangular groove in the side of the stage-plate, in which it slides forward and backward; and a thin plate (b and f)longer than the preceding and a little narrower, its bevelled edges sliding laterally in a V-shaped groove on the inner edges of the anterior and posterior bar of the frame just mentioned. The project-ing ends of this plate serve as hand-rests in dissecting. Three small screws are set in it as stops for the slide, and the knobs by which it is moved are bored in the centre as sockets for the stage forceps. Its central opening is of the same size as the larger opening in the stage-plate, and in this rests loosely a circular piece of glass (c)with a central opening (d), on which a dish may be set in examining small alcoholics. This apparatus, when well fitted and smoothly ground, works with a nicety and precision scarcely, if at all, inferior to that of the geared movement. An ordinary slide in position is shown at e.

This stage was made October, 1890, to my order and from my drawings, by the McIntosh Optical Company of Chicago, from whom I learn that it is now furnished with many of their own instruments, being adapted to the round stage-plate of their Microscopes by placing under it a thin false stage-plate which bears beneath a socket that slips into the central opening of the stage."

Introduction to the use of the Polarization Microscope in Histological Investigations.\*-This little book is intended by Dr. H. Ambronn to assist those who do not possess the physical and mathematical training neccessary for understanding the more advanced works on the same subject such as those of Valentin † and Nägeli. † To the mineralogist and petrologist the polarization Microscope has become an indispensable requisite, but even at the present time it is very little used by histologists. This is mainly due to the wide-spread idea that a very thorough knowledge of physical and mathematical optics is necessary in order to be able to work with this instrument. The author's aim in the present work is to give quite an elementary treatment of the subject with the hope of turning the attention of histologists to these methods of investigation. The book is thus only intended to be an introduction to the larger manuals, and accordingly only the simplest explanations are given and all mathematical formulæ are avoided.

The first four chapters of the book are devoted to an elementary discussion of the undulatory theory, the phenomena of polarization, double refraction, the interference colours between crossed nicols, and the use of the gypsum plate in determining the position of the axes of elasticity.

<sup>\* &#</sup>x27;Anleitung zur Benutzung des Polarisationsmikroskops bei histologischen Untersuchungen,' Leipzig, 1892, 59 pp., 27 figs., and 1 coloured plate. † 'Die Untersuchung der Pflanzen und Thiergewebe in Polarisierten Lichte,'

<sup>† &#</sup>x27;Die Untersuchung der Pflanzen und Thiergewebe in Polarisierten Lichte,' Leipzig, 1861.

<sup>&</sup>lt;sup>†</sup> Die Anwendung des Polarisations-apparates auf die Untersuchung vegetabilischer Elementartheile, Leipzig, 1863.

In the two succeeding chapters examples are given of the application of the methods upon cylindrical and spherical objects belonging to the vegetable kingdom, such as starch-granules, &c. The subject of pleochroism is then touched upon, and lastly a brief description is given of the methods of investigation in convergent light.

### (2) Eye-pieces and Objectives.

Spencer & Smith's Aplanatic Eye-piece.\*—Dr. M. D. Ewell writes :— "I have recently purchased from Spencer & Smith, of Buffalo, N.Y., a 1 in. positive eye-piece, which is so far superior to anything I have ever before used (and I have a large assortment), that I feel justified in calling the attention of microscopists to it. In common I suppose with the majority of workers, I, for a long period, paid no special attention to my eye-pieces, which, however, happened to be good ones, but centered my attention upon the objective and stand. I have long supposed that no available use could be made of any eye-piece for the micrometric purposes except in a limited portion of the centre of the field, never exceeding one-half thereof.

With the eye-piece in question I find sensibly equal amplification and no distortion, almost to the extreme edge of the field. In this respect it far surpasses the Ramsden and Huyghenian eye-pieces.

I find also that with it the definition, which I always test on a podura, is much improved, and that it is good almost to the extreme edge of the field, and this without any new adjustment of the focus. Altogether this eye-piece, which for want of a better name I shall call "Spencer & Smith's Aplanatic Eye-piece," is in my judgment a distinct advance over existing eye-pieces. I have ordered another one for my Zentmayer filar micrometer, and propose to use it hereafter in my micrometrical work in preference to those heretofore used."

New Objectives.<sup>†</sup>-Mr. H. L. Tolman remarks :-- "Among the new objectives recently made which are deserving of note are two, a 1/5and 1/8 both dry, of 150°, by Spencer & Smith, or as the firm used to be known, H. R. Spencer & Co., of Buffalo. These objectives are on a new formula, and for flatness of field, freedom from colour, and sharp definition they rank very high. In fact the robust images they give so much resemble in character those of the Zeiss apochromatics that they They work easily through a No. 2 cover, would be indistinguishable. and of course have cover correction. Perhaps I am rather an enthusiast in favour of Spencer's work, but without prejudice to any one else, I say without hesitation, I never have seen better dry glasses than these. Ι believe there is none of the Jena glass used in them, but the chromatic aberration is most exquisitely corrected, and it is gratifying to know such correction can be made without the necessity of using the as yet unproved kinds of glass."

Magnifying Power of Objectives.<sup>†</sup>—Mr. H. L. Tolman offers the following remarks on this subject:—" The question of how much a given objective will magnify has always been an important, but difficult one to answer, and every assistance offered toward solving it is worthy of atten-

\* Amer. Mon. Micr. Journ., xiii (1892) p. 103.

‡ Tom. cit., pp. 93-4.

† Tom. cit., p. 98.

# 546 SUMMARY OF CURRENT RESEARCHES RELATING TO

The distance of ten inches has been assumed as the proper interval tion. between the objective and eye-piece, that being the average focal length of the normal eye, but where to measure from and to is not so easy to ascertain. Some opticians estimate from the end of the tube of the Microscope, others from the outside of the back lens of the objective, others from a point midway between the different lenses of the objective. and still others from the front end of the latter. The difference between these two extremes is fully two inches, which may cause a difference of 20 per cent. in the results. Still others choose the point where parallel rays sent through the lens from the front would come to a focus, called the posterior principal focus, and one or two others the posterior conjugate focus, still higher up the tube, a point where rays meet which emanate from another point in front of the objective, at a distance such that the size of the object and image are made equal. This is an easily established place, but a theoretical consideration of the optical principles involved shows that the only proper position from which to measure the tube length, is from the posterior principal plane of the objective. In a simple lens this is easily ascertained, and in a very thin lens can be called the centre of the lens; but in a complex combination where the distance from the front of the front lens to the back of the back lens is sometimes two inches, the exact point from which to estimate tubelength becomes important. These principal planes in nearly all converging lenses are situated inside the objective at different distances from the centre of the combination, depending on the power of the lens and the way in which the corrections are made. In two-system objectives where the magnifying power is effected nearly equally by both systems, the principal planes are near the centre of the systems, while in some high power objectives they may cross one another, the posterior plane being in front of the anterior. The principal foci anterior and posterior of a lens are also two important points to know, and when these four data are given they are all that are necessary for a discussion of the properties of a lens.

In the last Journal of the Royal Microscopical Society,\* under the head of Measurement of Lenses, Prof. S. P. Thompson has given a very exhaustive and able article on how to ascertain this point or plane from which the 10-in. tube-length is to be measured. The instrument he uses is very complex and expensive, but the measurements can be made, except for high-power objectives, with a near approximation by any one with a little mechanical ingenuity. The principle of the mechanism is as follows :- The objective to be tried is placed in a horizontal position, and some point on it, either the front end or some point on the side, is selected as a zero-point for all measurements, a beam of parallel rays is sent through the lens from the back, and the distance from the zeropoint to the focus  $F^1$  of these rays measured. The lens is reversed, and the focus  $F^2$  of the rays issuing at the back is measured from the zeropoint. Then two small glass micrometers with coarsely ruled lines are placed one in front of the other behind the lens, and moved by a screw until the image of one micrometer is seen in focus on the other, and the lines superimposed. The distance of these micrometers from the zeropoint is measured. We have now all the data for calculating the

\* Feb 1892, pp. 109-135.

principal planes. It is a well-known optical principle that when an image of an object as shown on the screen is found to be the same size as the object, the distance between the two will be four times the focal length of the lens. In the present instance let F represent the anterior principal focus, and  $F^2$  the posterior principal focus, obtained as above,  $S^1$  the anterior conjugate focus where one micrometer was placed, and  $S^2$ the posterior conjugate focus where the second micrometer was placed. Then the distance  $S^1 S^2$  is equal to four times the focal length, plus the distance between the two principal planes, because an objective is not

equivalent to a bi-convex lens. To get the difference between the principal planes it is only necessary to subtract the distance  $S^1 S^2$  from twice the distance between the anterior and posterior principal foci.

Now, to find where these planes are, take the distance from the front focus or anterior principal focus to the micrometer, this is the true focus, and measure it backwards towards or along the objective, it will fall in the tube, perhaps a quarter of an inch from the front end; this is the first principal plane. Then measure from the back focus or posterior principal focus to the other micrometer, and that will be the distance to lay off on the tube from the back focus toward the front end of the objective; mark it on the tube, as it is the much-desired posterior principal plane from which the 10 in. is to be measured. To ascertain all this practically perhaps seems hard, but it is not very difficult to get very close measurements. A low-power objective should be chosen, and laid on a piece of cork along the edge of a board or table. For micrometers, take a stiff piece of writing-paper, and rule a series of lines 1/50 in. apart, using a Brown and Sharp's steel rule as a guide; cut this paper across the lines so as to make two micrometers, thus securing uniformity in the lines, as if each micrometer was ruled separately the lines might not agree. Dip these papers in oil or hot paraffin to make them transparent, and mount in a slit in a piece of cork at such a height as to be able to see them Take all measurements through the objective. with a pair of callipers, and lay them off on a rule. One ought to be able to get the principal planes within 1/50 in., and five times this would only make an error of 1 per cent. in the tube-length."

Fig. 59.



New Arrangement for the Quick Change of Microscope Objectives.\*—Herr H. Boas describes a simple form of adapter which can be applied to any Microscope model, even to small

\* Zeitschr. f. Instrumentenk., xii. (1892) 162-4.

instruments, in which the coarse-adjustment is by sliding socket. It is intended to replace the popular revolver which is so inconvenient when any manipulation of the preparation has to be made on the stage.

In fig. 59 the new adapter is represented 4/5 of its actual size. The adapter proper *a* is screwed in the ordinary way upon the body-tube instead of a system, while the objective to be used is provided with a connecting ring, the front edge of which fits exactly into a groove in the plate S. This plate is open on one side, in order to admit the neck of the connecting ring. A steel spring f, in the shape of a horse-shoe, screwed into the interior of the main-piece, serves to press the ring firmly into the groove. The plate S is attached to the main-piece *a* by four screws in such a way that an exact centering of the ring with the objective attached to it is insured.

Paper for Cleaning the Lenses of Objectives and Oculars.\*—The so-called Japanese filter-paper (the bibulous paper often used by dentists when filling teeth) is recommended for cleaning the lenses of oculars and objectives. It is said to be more satisfactory than cloth or chamois, because dust and sand are not present, and its bibulous character makes it very efficient in removing liquid or semiliquid substances. Use it for removing immersion-fluid from objectives, cloudiness or dirt from eyepieces, glass slips, or thin glass. Water, glycerin, or other fluids can be removed. Another recommendation is its cheapness.

# (3) Illuminating and other Apparatus.

Use of Polarization-Photometer.<sup>†</sup>—Dr. S. Czapski discusses the arrangement of the sections in the polarization-photometer so as to obtain achromatism of the bounding line without achromatism of the calcite prism. He explains how necessary it is in the use of photometers of any kind that the faces of which the brightness is to be compared should have a sharply defined line of boundary.

In polarization-photometers the usual arrangement consists in producing by double refraction two images  $a_o a_e$  and  $b_o b_e$  respectively, of two apertures a b situated at one end of the apparatus at a determined distance d apart. The magnitude of the double refraction bears such a relation to the distance of the apertures, that  $a_o$  and  $b_e$  are exactly adjacent, as seen in fig. 60.

Since refraction is accompanied by dispersion, the edges of the images  $a_o$  and  $b_e$  have coloured seams, and since the dispersive power of calcite is essentially different for the ordinary and extraordinary ray, simultaneous achromatism of both images is impossible. The most that can be done in this direction is to use, for the partial achromatism of the calcite prism, a flint glass with dispersive power lying between that of the ordinary and extraordinary ray in calcite, and thus to distribute the unavoidable chromatism uniformly over both images. In this case accordingly there remains a primary coloured seam on both boundary lines, which renders judgment of the brightness of the two fields difficult.

The author proposes to remove this coloured seam by the very simple but ingenious device of arranging the apertures, not sym-

<sup>\*</sup> Amer. Mon. Micr. Journ., xiii. (1892) pp. 99-100.

<sup>†</sup> Zeitschr. f. Instrumentenk., xii. (1892) pp. 161-2.

metrically, as in fig. 60, but in such a way (fig. 61) that the boundary lines fall in the plane of the double refraction. By this means the ordinary image  $A_o$  of the one aperture is formed above the extraordinary image  $B_o$  of the other, and no coloured seams appear on the boundary lines, for the simple reason that no deviation nor dispersion takes place in the direction at right angles to these lines.



For the same calcite prism, in order to obtain the right effect, the apertures A B (fig. 61) must be chosen smaller or closer together than the apertures a b (fig. 60). But since in this new arrangement no achromatism (and consequent diminution of deviating effect) of the calcite prism is necessary, the deviation by double refraction can be made essentially greater than in the first case, so that apertures really larger and farther apart than in the ordinary apparatus can be used.

The arrangement here described can be applied to all apparatus in which the juxtapos tion of the ordinary and extraordinary images of two apertures is effected by double refraction.

A Revolving Table.\*—The following is an account of Mr. F. L. Morton's device :—

I. Saw out a circular board 18 in. in diameter, and ornament the edge if you choose. On the under side, and about 2 in. from the edge, place four castors equidistant, and have the board rest firmly on them. In the centre of the under side bore a hole nearly through the board and insert a piece of brass tube. Stain or paint the board at pleasure.

II. Cut a piece of thick pasteboard 18 in. square and to the centre of it fasten a block  $2 \times 2$  in. by 1 in. thick, letting a brass pin stick up an inch from its centre, having it of such size as to work loosely in the tube.

III. Place the second apparatus on a small stand or table, adjust the first apparatus over it so that the pin will fit into the tube, revolve the top part upon the pin as a centre guide and upon the castors as lateral supports. If the castors are noisy make a track with felt for them to run on.

\* Amer. Mon. Micr. Journ., xiii. (1892) p. 120.

#### 550SUMMARY OF CURRENT RESEARCHES RELATING TO

A simple Geometrical Indicator for the Microscope.\*-Dr. Pietro de Vescovi describes a new form of indicator which has the advantage over those in general use of great simplicity and general applicability to any It consists of a simple system of four straight lines (see Microscope. fig. 62), traced on the stage of the Microscope, which virtually intersect in the projection of the optical centre of the field of view in such a way that each line is at 45° to the next.



FIG. 62.

In order, with this system of lines, to mark and recover the position in a preparation of an interesting point, the slide is held firmly or pressed against a projecting piece on the stage and a light mark is made with ink at three points on three adjacent lines of the system. The point of interest can at any time be again brought into the field of view. by bringing the three points marked upon the slide upon the three adjacent lines of the system.

The author would recommend Microscope-makers to engrave this system of lines on the stages of their instruments in white and red alternately on a black ground.

FRIEDRICH, P.-Eine Heizvorrichtung des Mikroskopes zu bakteriologischen Untersuchungen. (On a Heating Arrangement for the Microscope in Bacteriological Investigations.)

Arb. a. d. k. Gesundh.-A., VIII. (1892) No. 1, pp. 135-9.

\* Zool. Anzeig., xv. (1892) pp. 203-5.

### (4) Photomicrography.

Photographing Bacteria.\*—Sir H. E. Roscoe and Mr. J. Lunt describe a method of photographing bacteria, which they say is very

simple and requires no special apparatus or "microscopic accessories." The arrangement of the apparatus is shown in fig. 63.

A common duplex paraffin oil lamp was the source of illumination. As methylviolet was the stain employed and as this transmitted actinic rays of light a screen was spectroscopically adjusted to the stain employed, and a weak solution of potassium bichromate was found to serve the purpose admirably. The stained bacteria appear black on a bright yellow background. The photographic plates employed must, therefore, be sensitive to yellow light.

Abbe's condenser was used, without diaphragm, and was focused rather farther from the object than for ocular ex-A simple clip amination. stage was employed without mechanical accessories. The microscopic preparations were ordinarily obtained from young pure cultures, thus securing cells full of protoplasm, which stain deeply, an essential for actinic contrast on the photographic plate. Canada balsam in xylene was uniformly employed as a mounting medium. The lenses used were Leitz' 1/12 oil-immersion for 740diameters, Zeiss' D for 370, and Zeiss' A for 100 and 50 diameters. No eye-piece was used, nor was there any lens in the camera, which was connected to the tube of the Micro-



scope by a horizontal dark box extension. Edwards' isochromatic plates \* Phil. Trans., 182 B (1892) pp. 642-4. were used throughout. For the image shown by the immersion lens the exposure was about  $1\frac{1}{2}$  minutes, with correspondingly shorter exposures for Zeiss' A and D.

#### (6) Miscellaneous.

Ink for Writing on Glass or Porcelain.\* — The 'Rundschau' (Prague) gives the following:—Dissolve in the water-bath 10 parts bleached shellac and 5 parts Venice turpentine in 15 parts oil of turpentine. Incorporate in the solution 5 parts of lamp-black. Socalled diamond ink for writing on glass is a compound of fluoric acid and barium; the latter has no effect, it being simply a white powder to give body to the acid. The ink can be used with a rubber hand-stamp, and it should be allowed to remain fifteen minutes, when the barium will brush off, leaving the design on the glass.

Spherical Aberration-Apochromatic Objectives. † - Mr. Lewis Wright writes as follows : " Allow me to draw attention to another subject which vitally interests English microscopic opticians-viz. the production of apochromatic objectives. Though some of them have managed to secure a little supply, others are painfully aware that before the use of fluorite was allowed to become public, all the known available material had been secured by the firm of Zeiss at Jena; and the difficulty of getting material experienced by some of our best makers is a formidable obstacle to optical improvement, and tends to artificially keep up the prices. From an American periodical lent me by Messrs. Watson and Sons, I see that Prof. J. Brun has recorded in the 'Journal de Micrographie' the success of M. Albert Brun in producing by chemical synthesis what is called 'artificial opal,' and which is stated to have almost exactly the optical properties of fluorite, but to be harder, more homogeneous, and better suited for optical working. It is also stated that the process produces pieces large enough to be conveniently used in the manufacture of optical instruments. But this is the point. Prof. Brun apparently quotes from 'Archives des Sciences Physiques et Naturelles,' of Geneva, June 1891, and further quotes from it, or else states himself, that ' the house of Carl Zeiss, at Jena, has acquired the right to manufacture and to use this artificial opal for optical purposes.'

I think microscopical opticians have a right to ask what this means. It may mean no more than that the Jena firm has purchased a right to use this newly-available material, which is fair enough; indeed, I am such a baby as regards patent law, that I am not even sure whether or not a really international monopoly can be thus bought and sold. But if, as on its face appears, it is here meant that the German house has also effected a monopoly of this artificial process, or attempted to do so, and thus to obstruct the fair play of competition and march of improvement, the sooner such a peculiarly German method of business is generally understood by microscopists the better. I sincerely trust the affair is one of only clumsy translation, and that some one authorized to speak on behalf of Messrs. Zeiss will be able to dispel any such suspicion, which would assuredly do them more harm in the long-run

<sup>\*</sup> Amer. Mon. Micr. Journ., xiii. (1892) p. 110.

<sup>†</sup> English Mechanic, lv. (1892) pp. 220-1.

than any possible temporary advantage to be secured in such a peculiar way."

Dr. S. Czapski,\* in reference to Mr. Wright's letter, writes :-"Having read Mr. Lewis Wright's extraordinary letter (33427) in No. 1414 of the 'English Mechanic' on the subject of the employment of fluor spar in apochromatic lenses and the substitution for it of artificial opal obtained by Mr. Brun's method (vide 'Archives des Sciences Physiques et Naturelles,'t. xxv. No. 6), I will attempt to satisfy his curiosity by the following statements.

As regards fluor spar, Mr. Lewis Wright is labouring under a great delusion in assuming that before the use of fluorite was allowed to become public, all the known available material had been secured by the firm of Zeiss at Jena. The contrary may be said with more truth. The firm of Zeiss possessed but a very scanty supply at a time when, even previous to Mr. Koristka's groundless attacks in the 'Journal de Micrographie,' the fact that fluor spar was being used in the apochromatic lenses had been published three times in consequence of information supplied by the firm of Zeiss.

The latter were completely prepared to produce their future apochromatic lenses without having recourse to fluor spar, which by no means constitutes the condition sine quá non for the production of apochromatic objectives, excepting, of course, in the case of such opticians who can only produce them by slavishly copying existing systems. As, however, the firm became eventually possessed of a considerable quantity of clear material the employment of fluorite in their apochromatic lenses was continued.

But as regards the artificial opal of Mr. Bran, it is, in the first place, quite erroneous to treat it as a substitute for fluor spar, for though its refractive index is comparable to that of fluor spar (for fluor spar,  $\mu D = 1.434$ ; for opal,  $\mu D = 1.450$ ), yet the relative dispersive powers are markedly different—viz.  $\frac{\mu D - \mu \sigma}{\mu D - 1} = \frac{1}{95 \cdot 4}$  for fluor spar, and  $\frac{1}{67 \cdot 2}$ for opal. With the latter the dispersion is, therefore, even greater than that attainable with phosphate glasses. For this reason the firm of Zeiss wrote about eighteen months ago to Mr. Brun:—

That they had abandoned their original hope of a glass to be produced by Mr. Brun of exceptionally low relative dispersion, and that there remained, therefore, only an exceptionally low refractive index. It became now a question of determining in what department of practical optics a substance possessing these properties might yield particular results, which were not obtainable with other glasses. For as in any case the production of your glass will involve greater difficulties, and probably also higher costs, than is the case with silicate glasses (which also admit of the value of  $\frac{\mu - 1}{\Delta \mu}$  being reduced to about 65), and as the production of *large* pieces (e.g. for telescopes and large photographic

objectives) is very likely to cause considerable—perhaps insurmountable —difficulties, it would become necessary to determine those cases in which its characteristic property, the very low refractive index, con-

<sup>\*</sup> English Mechanic, lv. (1892) p. 287.

# 554 SUMMARY OF CURRENT RESEARCHES RELATING TO

stitutes an advantage sufficiently great to compensate for the difficulties of its application.

We have, with respect to this question, had to investigate the subject by special calculations, no one being in a position *a priori* to answer it. These investigations have, however, hitherto not led to any *positive* results. It remains, in fact, still highly problematic whether there be any problem in practical optics the solution of which may be approached by the existence of a glass possessing the characteristics of the artificial opal.

Nevertheless, the substance in question interests us in a high degree. We recognize in its appearance a valuable attempt to extend the range of optical means, and an incitement for new studies in the production of artificial glasses. As we ourselves, in conjunction with Dr. Schott, have worked in this direction for a period of ten years, and intend to continue our researches, the outcome of *your* experiments is indeed full of interest to us, quite irrespectively of the question whether your material affords a means of immediate practical application or not. For this reason we entertain a wish of at least rendering your researches available for continuation in our own experiments in glass smelting.

We make to you, therefore, the following proposition :--

We offer you to defray the expenses of your past experiments (... francs), provided you agree to communicate to us the composition of, and manner of, fusing your glass with such exact and complete directions as to enable Dr. Schott to reproduce it in his laboratory. We further stipulate that you place in our hands a few samples of the material obtained by yourself enabling us thus to at once proceed with experiments on its properties; that you authorize us, or Dr. Schott, to compound your glass according to your directions, in order that we may obtain material for practical *experiments*, and also to utilize the process indicated by you in researches aiming at the production of other similar glasses.

It is to be understood that you retain all rights as to priority, in particular the right of publishing your experiments, inclusive of the composition of your glass and the mode of fusing it, before we publish anything on the subject or make any attempt to utilize the glass commercially. It is, however, to be agreed that you promise to suspend such communications to others on the compositions and production of the glass *for one year*, in order that, in the meantime, we may find sufficient time to exhaustively investigate the matter.

As this proposition agrees entirely with the first offer which you made in your letter of the 28th Sept., excepting only that in the present proposition we cede to you further rights which we do not consider ourselves justified in claiming for ourselves, we assume that the above will meet with your approval.'

And after having received the desired communications from Mr. Brun, the firm of Zeiss wrote again :--

'We should consider it very inappropriate to any longer insist upon your continuing to keep secret the general results of your studies—i.e. the artificial production of amorphous silica and the establishment of its optical character. These results are of such great scientific interest that you should, in order to establish your priority, forthwith publish them. Our interest in the continuation of your work would thereby not be affected in the least. In fact, had even those results been published by you previous to the formulation of our agreement, the same proposal would have been made by us. We only stipulate that you should, for the present, exclude your personal experience regarding methods and means from publicity, and that in your publication you mention that the firm of C. Zeiss, in Jena, has undertaken to render your experiments, if possible, available for the requirements of practical optics. (The numerical values of  $\mu$  and  $\Delta \mu$  very nearly agree with the result obtained by spectrometric measurement by Dr. Abbe years ago with respect to several varieties of natural opal.)'

These extracts will show Mr. Wright how much truth there is in his statements with respect to the monopoly of the German house. I emphatically endorse his remark 'the sooner such a peculiarly German method is understood by microscopists the better.' True, if he means the better for the firm of Zeiss.

In order, however, that Mr. Lewis Wright may be freed from any vestige of a doubt, the firm of Zeiss offer to place at his command all communications which they have received from Mr. Brun, and they offer him a premium of 1000m. if he will pledge himself to supply within the period of one year 160 grammes of optically useful opal produced after the method of Mr. Brun.

If Mr. Wright be not equally a 'baby' in technical processes, as he confessedly is in matters pertaining to patent law, he will find it an easy task to merit the premium offered to him, and to enrich the stores of technical optics by such a valuable material."

To this letter Mr. R. Kanthack adds the following :-- "The above letter of Dr. Czapski (which, by the way, is a translation, and should, in fairness to the original writer, be read as such) will no doubt be sufficient to remove Mr. Lewis Wright's doubts; but it may not be uninteresting to him to hear that within the last eighteen months respectable quantities of fluorite were offered me in my capacity as agent of the firm of Zeiss. The fact, however, that the price offered almost ridiculed [sic] the price expected -so much so, that in one case it was thought more profitable to utilize the fluor spar for garden decorations-agrees but imperfectly with the allegation that the firm of Zeiss is bent upon securing all existing fluorite mines. Summing up Dr. Czapski's explanatory statements, it would appear that for the construction of apochromatic lenses fluorite is 'useful,' but mathematics 'indispensable.' I may here mention that it is only due to want of appreciation of the mathematical principle of apochromatism that the terms 'semi-apochromatic lenses' and 'apochromatic glasses' (the latter as applied to the raw material produced by the Abbe-Schott process) retain their scientific sound, when in reality they can claim no more meaning than that attachable to trade advertisement."

# B. Technique.\*

Zimmermann's Botanical Micro-technique.<sup>†</sup> — We have here a much-needed handbook of microscopical preparations, chemical reactions,

\* This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes;
(4) Staining and Injecting: (5) Mounting, including slides, preservative fluids, &c.;
(6) Miscellaneous.

<sup>+</sup> 'Die Botanische Mikrotechnik,' von Dr. A. Zimmermann, Tübingen, 1892, **x**. and 278 pp. and 63 figs.

and methods of staining. It is divided into three sections. The first treats of general methods, the examination of dried plants, maceration, swelling, clearing, methods of fixing and staining, &c. The second part discusses micro-chemistry, and the reagents for the various substances found in the vegetable kingdom. In the third section we have a detailed treatise on the methods of investigation for the cell-wall and the various inclosed substances, and for the differentiation of the protoplasmic A supplement treats of the methods of investigation of constituents. bacteria; and the whole concludes with a copious bibliography and a The wood-block illustrations, both those which belong verv full index. to the first part, and those which depict the appearances presented under the Microscope by the use of reagents, are exceedingly good and clear, most of them new.

# (1) Collecting Objects, including Culture Processes.

Automatic Device for Rolling Culture Tubes of Nutrient Agar-Agar.\*-Prof. G. F. Atkinson describes a process which he has found successful for rolling culture-tubes of agar-agar under a continuous shower of cold water instead of using ice for the purpose. The apparatus consists of a tin jacket, with rectangular perforations and bristling with "paddles," which grasps the tube, and upon which the stream of water is so directed that it furnishes not only the motive power for whirling the tube, but also the cold bath to solidify the agar-agar. The mode of making and using the jacket is described in detail.

Bacteriological Technique.<sup>†</sup>—Dr. G. H. F. Nuttall says that in many cases the ordinary loop made of platinum wire does not work well because it bends, and advises the use of a stiffer instrument such as he has invented. This resembles a small spear, is made of wire 1 mm. thick, beaten out at the free end into a triangular flattish extremity, in the centre of which is a teardrop-shaped perforation (see fig. 64).

FIG. 64.

(2) For examination of drop cultivations the author advises the following method which is very convenient and less tiring to the eyes than that in vogue, as by its adoption focusing is much facilitated.

A thin black ring composed of lampblack and blood-serum is run round a cover-glass by means of the turntable. The cover-glass is then sterilized in the usual manner and the drop placed in the middle of the ring. As it is easy to focus the ring and as the organisms lie in the same plane, the latter are easily found by merely pushing the preparation along.

(3) Test-tubes may be closed with flat discs of paraffin made by

- \* Bot. Gazette, xvii. (1892) pp. 154-6 (1 pl. and 1 fig.).
  † Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 538-40 (2 figs.).

punching out pieces from a paraffin plate. The discs should have a diameter a little larger than that of the test-tube. They may be sterilized in sublimate and kept covered up ready for use. When required they are warmed a little and the cotton-wool plug having been burnt and removed, the disc is inserted and jammed into the opening so that



the tube is now hermetically sealed. In case tubes thus prepared are to be kept at a higher temperature the cap is perforated in order to allow the gas or air to escape, and the hole afterwards sealed up with fresh paraffin.

(4) Blood-serum is obtainable very satisfactorily in the following manner. The flasks (fig. 65) may be made of any size and to hold 10-100 cm. or more. The broader of the two tubes is plugged with cotton-wool, while the other is drawn out to a fine point and closed.

The artery, from which the blood is to be drawn, having been exposed is tied in two places. The ligature farthest from the heart is drawn tight, while the proximal ligature is merely loosely tied; just above this the artery is clamped. An opening is then made between the two ligatures and the thin tube of the flask (its end having been broken off) is inserted into the artery, and the loose ligature drawn tight so that it holds both tube and artery. The clamp is then released. When sufficient blood is obtained the artery is compressed, and the apparatus having been withdrawn the fine tube is sealed up in the flame. In this way 2–3 flasks full may be obtained from each animal, and with a strong probability that they will be sterile. After coagulation the serum may be withdrawn with a pipette.

Preserving Malaria Parasites alive.\*—In a case of typical tertian ague, Dr. O. Rosenbach put a leech over the spleen; this died in 48 hours and numerous dead plasmodia were found therein. Two other leeches

\* Berlin Klin, Wochenschr., 1891, No. 34. See Centralbl. f. Eakteriol. u. Parasitenk, x. (1891) p. 806. were applied some hours before the beginning of an attack, and one of these, opened 24 hours later, was found filled with red corpuscles inclosing living plasmodia and mobile pigment; the other leech was opened 48 hours after sucking, and the appearances seem to have been quite similar. A leech applied 24 hours after treatment with quinine was found to contain a few shrunken plasmodia and a little immobile pigment.

It would seem therefore that the malaria parasites can be kept alive within the leech for at least 48 hours, and this retention of life suggests that the leech might be employed for studying the lifehistory of the plasmodia. It would be satisfactory to ascertain if the blood drawn by the leech were capable of infecting other animals.

The author suggests that human blood rendered artificially coagulable by leech substance might be used as a cultivation medium for malaria parasites.

Tubercle Bacilli and other Pathogenic Micro-organisms found in the Sputum and Lung Cavities.\*-Mr. S. Kitasato obtains pure or approximately pure cultivations of tubercle bacilli from the sputum by making the patients, after having well washed out their mouths, expectorate into capsules filled with sterilized water. The sputum must be coughed, not merely hawked up. The selected lumps are then washed ten times successively in so many vessels filled with steri-Cultivations are then made on agar and often these lized water. are quite pure. It was remarked, however, that cultivations from the sputum differ at the outset of their growth from those obtained from tuberculous organs. For the first two weeks they appear as circular white opaque flakes on the surface of the agar, and the colonies are furthermore distinguished by being moist, smooth, and shining (almost like colonies of white yeast) while those from the organs are dry, dull, and wrinkled.

By about the fourth week these differences disappear, the two sets of colonies becoming indistinguishable.

The author then goes on to state that most of the bacilli in the sputum and in the contents of lung cavities are dead, although microscopically they are exactly alike. This was proved both by cultivation and injection experiments.

From observing the constant association of other bacteria and their presence in considerable numbers, the author concludes that these other micro-organisms exert some influence on the disease, but to what extent is uncertain.

**Preparation of Sterile Gelatin Tubes.**†—In their researches on the chemical bacteriology of sewage, Sir H. E. Roscoe and Mr. J. Lunt prepared gelatin tubes in a manner which they say is simpler and shorter than that generally adopted. The test-tubes, 5 or 6 in.  $\times \frac{3}{4}$ , are first washed and set up on end to drain, and then heated to 150° for an hour. Pure cotton-wool is placed in a steam sterilizer and subjected to a current of wet steam for two hours, and afterwards dried in the hot-air sterilizer by heating to 150° for half an hour. This

\* Zeitschr. f. Hygiene, xi. (1892) pp. 441-4.

† Phil. Trans., 182 B (1892) pp. 662-4.

method gives a whiter wool without brittle and partly charred thread, and by it tubes can be prepared in one day. When about half a gross of tubes have been plugged they are replaced in the hot-air sterilizer and raised to  $150^{\circ}$  for an hour.

To prepare sterile nutrient gelatin, one pound of lean beef is finely minced, and a litre of tap water is poured over the mass; the whole is placed in the steam sterilizer for an hour and a half, and is then filtered into a large beaker containing 100 grm. of gelatin, 10 grm. of peptone, and 5 grm. of salt. The hot filtrate quickly dissolves the gelatin. The mixture is now placed in the steam sterilizer for half an hour, neutralized with potassium carbonate, or replaced in the sterilizer for another hour. The turbid fluid is now filtered into a large flask and distributed to the sterile plugged tubes; these are now steamed for fifteen minutes, and the steaming is repeated on the second and third day for ten minutes each day. This treatment is quite effective.

Before opening the tubes the tuft of wool was uniformly singed to burn up the dust and germs which might have fallen on the outside. In the preparation of sterile peptone broth the only difference is that the 100 grm. of gelatin are omitted. For sterile agar-agar 20 grm. of agar are used instead of 100 grm. of gelatin. Petri's dishes (shallow covered glass dishes about a decimetre in diameter and 15 mm. deep) are much more simple to work with and give less contamination from the air than the original glass plate and bell-jar method.

Investigation of Chemical Bacteriology of Sewage.\*—Sir H. E. Roscoe and Mr. J. Lunt adopted the following method for the isolation of anaerobic organisms. They devised a special form of cultivation flask (fig. 66) suitable not only for mixed cultures, but also for pure cultures, in which the organisms can be grown in an atmosphere of pure hydrogen.

The flask is furnished with a capillary tube e, sealed in at f, for the purpose of introducing hydrogen. A firm plug of sterile wool at g excludes foreign germs. When it is desired to sterilize the flask and its contents before the introduction of pure material or sewage, the fine jet a is sealed, and the opening c, for the introduction of the culture fluid and organisms, is protected by a sterile plug d. The whole is steamed for twenty minutes on two or three successive days, and is then ready for use. The plug d is carefully removed, and a few drops of sewage are introduced by a freshly drawn out capillary pipette, after which the tube is sealed at c. Pure hydrogen is now passed through the liquid by means of the capillary tube e, the gas issuing by the broken off end of tube a, immersed in water to shut off all communication with the air. After the gas has passed for half an hour and every trace of oxygen is expelled, the flask is hermetically sealed at h and b.

For the isolation of spore-forming organisms the following method was used; a few drops of sewage were introduced into a sterile broth tube by means of a recently drawn out capillary pipette, and the plug was replaced. The tube was then plunged into water at  $80^{\circ}$  for ten minutes; this suffices to kill all the full-grown bacilli, but is not

<sup>\*</sup> Phil. Trans., 182 B (1892) pp. 635-7.

sufficient to kill the spores. The spore-forming organisms may now be isolated by plate culture.



**Bacteria Harpoon.\***—Dr. Unna has devised an apparatus for fishing out bacteria from minute and particular colonies. It is called a harpoon and the idea of the inventor was to replace an objective by a needle. Hence the apparatus is intended to be fitted on to the Zeiss sliding objective-changer. The harpoon is constructed of a tube of metal, the proximal end being threaded to screw into the slide and the distal split in order that a needle may be inserted. The needle is fixed in position by means of a screw.

The instrument is manipulated in the simplest manner. First, it is necessary to ascertain if the search-lens and the harpoon are centered. This is done by means of a cross-thread ocular, and if a hole made by the needle-point, say, in the cultivation plate, centres accurately with the crossing-point of the threads, then the desired colony is placed in the exact position; the lens is exchanged for the harpoon, the point of the needle is screwed down on the colony; then having been screwed up the harpoon is removed, the inoculation made, and then the needle disinfected and so on.

It is obvious that the only difficulty in working this apparatus arises in connection with the centering of the search-lens and the needle, and this, as the author says, is for the microscopist a trivial affair.

Strauss' Method for quickly Diagnosing Glanders.<sup>†</sup>-Herr G. M. Finkelstein records a series of experiments made for the purpose of

> \* Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 278-80. † Tom. cit., pp. 433-8.

diagnosing the presence of glanders in horses by Strauss' method, a method which consists in injecting some of the suspected glandered tissue, or of cultivation from this into the peritoneal sac of male guinea-pigs. One of the principal results of this method is an affection of the testicle and its coverings, which is observable two to three days after inoculation. The skin of scrotum is tense, red, slimy; suppuration often occurs, and in the pus may be found the bacilli of glanders. The animals die in from four to fifteen days. Both the tunica vaginalis and the body of the testicle are affected.

The method would seem to be both easy and effectual for diagnosing glanders, for if the testicles of an animal become inflamed on the second or third day after intraperitoneal inoculation, the presumption would be that the disease is glanders.

The glandered material was derived either from the nasal secretion or from a piece of the submaxillary gland; these were either inoculated on some cultivation medium, or were rubbed up with bouillon or water and then used directly as an injection.

ARONSON, H .- Ueber die Anwendung der colloidalen Thonerde zur Filtration bakterienhaltiger Flüssigkeiten. (On the use of Colloidal Clay for the Filtration of Fluids containing Bacteria.) Arch. f. Kinderheilk., XIV. 1891, pp. 54-8.

#### (2) Preparing Objects.

Investigation of Structure of Pancreas.\*-Prof. C. J. Eberth and Dr. K. Müller used the pancreas of freshly killed animals. They preserved the organ in Flemming's or Rabl's mixture, or in Hermann's fluid; in Flemming's mixture, 1 per cent. solution of platinum chloride took the chromic acid. Good results were also obtained by the use of a 1/3per cent. solution of platinum chloride and Kleinenberg's picrosulphuric acid. The secondary nuclei were not well shown when fixation was effected with corrosive sublimate. The hardened glands were imbedded in celloidin or paraffin; the latter is better for those animals in which Care, however, must be taken the cells are smaller than in Amphibia. in making the sections. Hæmatoxylin with eosin, Platner's nucleusblack, Ogata's hæmatoxylic eosin, nigrosin and saffranin were among the staining reagents; Babes' safranin-anilin-oil is recommended.

Examination of Ectoparasitic Trematoda.<sup>†</sup> - Herr C. Dieckhoff finds that little is to be learnt from an examination of living Trematoda. The worms were generally killed by heated solution of corrosive sublimate, but in a few cases chrom-osmic-acetic acid or Müller's fluid was The objects were hardened in alcohol or Müller's fluid, and were used. all stained with picrocarmine. The serial sections were 0.01 mm. thick.

Preparation of Epiphytic Fungi.<sup>‡</sup> — For preserving the various parts of epiphytic fungi in their natural position while under examination under the Microscope, M. A. Gaillard recommends the following process. A drop of collodion is first of all dropped on to the fungus; but the

\* Zeitschr. f. Wiss. Zool., xxxv. Suppl. (1892) pp. 119 and 20.

 † Arch. f. Naturgesch., Ivii. (1891) pp. 247 and 8.
 ‡ Bull. Soc. Mycol. France, vii. (1891) pp. 233-4. See Bot. Centralbl., l. (1892) p. 75.

1892.

collodion of commerce does not form a sufficiently homogeneous pellicle; and the author recommends the following preparation:—gun-cotton 4 grm., 90 per cent. alcohol 10 grm., sulphuric ether 32 grm., castor oil 2 grm., lactic acid 2 grm. The lactic acid insures clarifying of the hyphæ. When the ether has evaporated, this collodion leaves behind a remarkably delicate pellicle, which must be carefully removed, carrying the fungus along with it. The cellulose is then again dissolved off by a drop of a mixture of 90 per cent. alcohol and ether, the glass slide heated, and a small piece of glycerin gelatin placed on the preparation; this at once liquefies, and incloses within it the fungus with all its parts in their natural position.

Preparing and Examining Hyphomycetes.\*-One of the simplest methods for examining spore-formation on aerial hyphæ is, says Dr. Unna, to use a cell-slide and to fill the cell with nutrient agar. When the medium has set, one-half is to be cut out and the fungi grown on the remaining half. Under these conditions pretty high powers may be used for examining the fungi and their growth. But a more effective and even simpler method is to grow the fungi in a test-tube and observe them in situ. On obliquely set media a cultivation track is made not only along the middle, but also along the edge where it joins the glass. This allows, especially if the glass be very thin, the cultivation to be well This method allows of numerous modifications; for example, the seen. cultivation having grown up, most of the medium may be got rid of by gently heating it, and then when liquefied, pouring it off, thus leaving only a thin layer, on which the growth is left behind. These "minimal cultivations" are very superior to all other methods for observing the various stages of growth, and a pure cultivation may be thus kept under observation for weeks or months.

The cultivations may be fixed and stained by the following method. The test-tube is filled in with the following mixture :— gelatin 1, liq. ammon. fort. and spirit equal parts  $25 \cdot 0$ , glycerin  $15 \cdot 0$ , distilled water  $35 \cdot 0$ . This moistens the fungi, drives off air-bubbles, renders the minimal cultivation quite transparent, and turns it into a permanent cultivation.

If the cultivation is to be stained, it is previously treated with a watery spirituous solution of some basic anilin dye; it is next washed with weak spirit, and then the glass vessel is filled up with salt solution or acetate of potash.

The cultivation is cleared up and fixed by treating it with alcohol and then with petroleum, to which a few drops of nitro-benzol have been added to prevent fluorescence.

While the natural growth of fungi is better observed in minimal cultivations, stained preparations are more conveniently obtained from slide cultivations, and very minute details are given by the author as to the manner in which he proceeds. The great difficulty appears to be to get rid of the nutrient medium, as this absorbs anilin pigments more easily than the fungi to be stained. However, by treating the cultivation with a 20-30 per cent. potash solution and warming moderately the medium is so softened that it may be squeezed away from the

\* Centralbl. f. Bakteriol. u. Parasitenk., x<sup>2</sup>. (1892) pp. 4-9, 40-44.

cultivation by means of a strip of oiled paper laid over the cultivation, the latter lying on the slide.

Preparations suitable for photographing are made as follows. A piece of agar cultivation is imbedded in celloidin and sectioned. The sections are then laid for about a minute in 5 per cent. caustic potash solution and after having been washed in water are immersed for five minutes or more in 5 per cent. acetic acid. The sections are then dried and stained for some seconds over the flame in phenol-fuchsin. They are again washed with water, and then partially dried with tissue paper. The sections are then dehydrated with anilin oil, and the latter having been removed with xylol the preparation is mounted in balsam.

By treating the fuchsin-stained sections for some seconds with a 1-5 per cent. solution of chromic acid or bichromate of potash, the contour of the fungi is said to be brought out more clearly, and the original colour to be darkened.

### (3) Cutting, including Imbedding and Microtomes.

Notes on Celloidin Technique.\*-Mr. A. C. Evclesheimer writes as follows :-- "The high value of celloidin as an imbedding mass is well known, † and its superiority over all methods requiring heat is unquestionable, yet, from the fact that its manipulation has been attended by many difficulties, it has not come into general use. During the past two years I have tried the methods recommended by various authors and have found none entirely satisfactory, especially where very long series were necessary. The results of my experience are embodied in the following method: the prepared plates or fragments are placed in an air-tight chamber; a 4 oz. salt mouth bottle being very suitable for this purpose. Pour into this bottle just enough ether-alcohol (equal parts acid, free sulphuric ether, and absolute alcohol) to cover the fragments. The ether-alcohol should be added until after occasional shaking no celloidin remains undissolved; this may take several days. It should finally possess the consistency of a very thick oil. The solution thus obtained may be labelled No. 4. No. 3 is obtained by taking two volumes of No. 4 and diluting with one volume of ether-alcohol. No. 2 by proceeding in a like manner with No. 3. No. 1 is a mixture of absolute alcohol and sulphuric ether in equal parts.

The saturation and final imbedding is accomplished thus: the object is transferred from 95 per cent. alcohol to solutions 1, 2, 3, 4, successively, in each of which it remains from a few hours to days, depending upon the size and permeability. For pieces of tissue 2 mm. in diameter twenty-four hours in each will generally suffice. For a large brain, e. g. that of a cat, a week in each will not be too long.

In imbedding, unless orientation is desired, the ordinary paper box is best. A thin plate of lead is placed in the bottom and the imbedding solution poured in. The object is taken from the same solution, and with needles wet in ether placed in the desired position. Fine needles may be passed through the box to support the object.

In hardening, the method given by Viallanes of immersing in chloroform is preferable, since the operations may be carried on with much

† See ante, p. 438. 2 Q 2

<sup>\*</sup> Amer. Nat., xxvi. (1892) pp. 354-7.

greater rapidity. An air-tight chamber should be filled with chloroform; a very wide-mouthed bottle will answer. After the mass is thoroughly hardened, which requires about twenty-four hours, it is removed, the paper cut from the sides, and transferred to 70 per cent. alcohol for a few hours.

It is now ready to fix for sectioning. Blocks are trimmed to fit the elamp of the microtome. Solution No. 3 is poured over the block; into this the celloidin block is pressed, after dipping the under surface in solution No. 1. Place in chloroform until hardened.

Reconstruction points are often very desirable. For this purpose the ordinary metallic imbedding box made of two L-shaped pieces, held in place by overlapping strips, is used. The ends and sides are perforated in as many places as desired by a very small drill. The holes should be so drilled that the silk threads which are drawn through run parallel.

After being drawn tightly they are cemented to the sides of the box by a drop of celloidin. Five or six cm. of the thread should be left hanging. The bottom of the box is made by fitting in a piece of heavy blotting-paper. The object is placed upon the threads in the desired position, and the imbedding mass poured in. As soon as hardened, the celloidin holding the threads is dissolved by a drop of ether. The loose ends are soaked in solution No. 2, which has been thickened by the addition of lampblack. The threads are then drawn through, leaving the lampblack adhering to the celloidin, thereby forming excellent reconstruction points.

For small objects, where reconstruction points are not needed, the following method may be advantageously employed. The heads are clipped from fine insect pins, which are then placed in handles in such a way that they may be easily removed. On these pins the objects are oriented in the desired position; the pins are then removed from the handles and fixed in a cork previously perforated by a somewhat larger pin. As fast as the pins carrying the object are inserted, the cork is replaced in the tube, which is filled with alcohol. A half-dozen fish or amphibian ova may be oriented on the same cork. If desirable to draw the objects in situ a piece of lead may be pinned to the cork, and the whole immersed in a small beaker of alcohol. The corks carrying the oriented objects are transferred successively to tubes containing the different solutions. When ready for final imbedding, a piece of porous paper is wrapped about the tubes and cork, and pinned. The cork is now removed, allowing the imbedding solution to fill the paper tube thus formed. A lead is fastened to the cork, and the whole placed in chloroform until hardened, after which the paper is cut from the mass and the pins drawn through the cork, when it is ready for sectioning. This method offers many advantages, in that several objects may be cut at the same time, drawings may be made after orientation, the objects are transferred from one solution to another more rapidly, &c.

In cutting, care should be taken that the knife is placed as obliquely as possible and kept constantly wet with 70 per cent. alcohol. For this purpose an ordinary pipette provided with a large rubber bulb is used. As fast as cut the sections are drawn back on the blade of the knife by means of a needle, and arranged in a single row until the blade is filled. To remove them a heavy paper spatula is placed directly upon the section to which it adheres, and may be drawn off the edge of the knife and transferred to the slide. By slight pressure, together with a rolling movement, the section is left in the desired position. Sufficient alcohol is kept on the slide to prevent drying, but not enough to allow the sections to float. When the requisite number have been arranged, they are covered with a strip of toilet paper which is held on the slide by winding it with fine thread. The sections being thus firmly held in position may be stained, &c. They should not be placed in absolute alcohol, but cleared from 95 per cent. in a mixture of equal parts of bergamot oil, cedar oil, and carbolic acid. When cleared the excess of fluid is removed by a piece of blotting-paper with gentle pressure, sections which are by chance loose are firmly fixed in position, the thread is now cut, the strip of paper rolled back, balsam and cover applied. If the object can be stained in toto, which is often the case, much time may be saved by the following method: The stained object is imbedded in the usual manner, but after hardening in chloroform, and removing the paper, the celloidin block is transferred to 95 per cent. alcohol for twenty four hours, then to carbolic acid\* or glycerin in which it becomes as transparent as glass.<sup>†</sup> The block is fixed in the usual manner.

Orientation is now accomplished with the greatest ease. In cutting, the knife is wet with the clearing medium given above. The sections may be arranged in serial order on the knife-blade until a slideful is obtained, when they are transferred, balsam and cover applied. By this method long series may be readily handled. Glycerin is used only when the mounting medium is glycerin. In this case the knife is wet with glycerin."

Taylor's Freezing Microtome.<sup>‡</sup>—Dr. T. Taylor remarks :—" This combination microtome is adapted to three methods of section-cutting.

The instrument is of metal screwed to a block of polished mahogany. There is a revolving table with graduated margin, in the centre of which is fitted a freezing-box having two projecting tubes, one to admit freezing water, the other an outlet for it. The water is supplied from the reservoir and carried off by means of rubber tubing which is attached to the metal tubes, the terminal end of the outlet tube being furnished with a small glass tube, by means of which a too rapid outflow of water is prevented. The tubes of the freezing-box are so arranged as to prevent their revolving with the revolutions of the table. When ether is used, the little brass plug in front of the freezing-box is removed and the rubber tubing detached.

In preparing to make sections, remove the freezing-box, and in its place substitute a cork which projects suitably, holding the object from which sections are to be taken, imbedded in wax or paraffin, at the

\* Bumpus (Am. Nat., Jan. 1892) advises the use of thymol.

† Since discovering this method of rendering celloidin blocks transparent, which was published in the Bot. Gaz., 1890, I have found that the clearing mixture given above answers the same purpose as the carbolic acid, but requires a little longer time.
‡ Amer. Mon. Micr. Journ., xiii. (1892) pp. 25-6. required angle to the blade of the knife. The cork is raised or lowered by means of a finely cut screw-thread.

The curved knife is about 5 in. in length and 1 in. in breadth, ground flat on the under side, and held in position by a binding-screw, after the fashion of several microtomes previously in use. A straight knife may be used if desired."

# (4) Staining and Injecting.

Double-staining of Sporigenous Bacilli.\*—Sig. L. Macchiati describes the mechanical details of a process which he has found useful for the double staining of bacilli containing spores. The following are its more important points. The microbes are picked out by a sterilized platinum needle, and placed in a drop of sterilized distilled water which is evaporated in a platinum dish over an alcohol flame. When quite dry a staining solution is added composed of 5 grm. carbolic acid, 20 grm. alcohol, and 0.8 grm. fuchsin, made up to 100 grm. with water. When this is boiled and evaporated, the spores take up a rose-coloured stain which is very persistent, while the colour can be entirely removed from the bacillus itself by absolute alcohol. The bacilli can then be stained in the ordinary way by gentian-violet or methylen-blue.

Carbol-methylen-blue Method.<sup>†</sup>—Herr F. Pregl advises the following modification of Kühne's methylen-blue method <sup>‡</sup> as it is shorter and less decolorizing than the original procedure. The sections stuck on slide or cover-glass are stained for 1/2 to 1 minute with carbol-methylenblue, with or without aid of heat. 'They are then washed for a short while with distilled water. Next they are immersed in 50 per cent. alcohol until they become pale blue with a somewhat greenish tinge, after this they are dehydrated in absolute alcohol, cleared up in xylol, and imbedded in balsam.

**Spore-staining.**§—Herr Foth says that the method suggested by H. Möller for staining spores is excellent. The process is as follows:— 1. Fixation by heat or absolute alcohol (2 minutes). 2. Removing fat, &c., in chloroform, 2 minutes. 3. 1/2-2 minutes' action of 5 per cent. chromic acid. 4. Staining with phenol-fuchsin over the flame for 60 seconds, once boiling. 5. Decolorizing in 5 per cent. H<sub>2</sub>SO<sub>4</sub>. 6. Contrast staining in saturated aqueous solution of malachite-green or methylen-blue, 30 seconds. By this method the spores are stained red and the bacilli blue.

Instead of chromic acid, chlorine water, eau de Javelle, or peroxide of hydrogen may be used; indeed, for many spores chromic acid is too strong, and is replaced by peroxide of hydrogen with advantage. Sometimes it is better to use anilin instead of carbolic acid with the fuchsin. Fixation by means of Loeffler's device, i.e. holding the cover-glass between thumb and finger over the flame, is just as safe and more advantageous than immersing the cover-glass in chloroform, though the latter procedure has its advantages.

- \* Malpighia, v. (1892) pp. 431-3.
- † Centralbl. f. Bakteriol. u. Parasitenk., x. (1892) pp. 826-9.
- ‡ See this Journal, 1890, p. 254.
- § Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 272-8.

Besides the mere staining of the spores the author had in view the object of ascertaining if the resistance of spores could be measured by means of staining. Numerous experiments were made with tetanus, anthrax, symptomatic anthrax, and other bacteria, but the results, as far as the end in view was concerned, were not successful.

Staining Micro-organisms of the Cuticle.\*-In preparing any portion of the outer skin (or its pathological derivatives), epidermis, hair, nails, scabs, &c., Dr. Unna first treats the specimen with a drop of acetic acid, then squeezing and flattening it out between two slides. This done, the slides are drawn apart and dried quickly in a flame; the fatty matters are next removed by running a few drops of ether-alcohol over them while held obliquely.

Then upon one slide are placed two drops of borax-methylen-blue (borax 1, methylen-blue 1, H<sub>2</sub>O 100) and this is covered with the other slide, and the two heated in the flame for 10-20 seconds. After this, the preparations are further decolorized or dried in the flame.

The author treats preparations deprived of fat and dried in the air with a drop of borax-methylen-blue solution, and a drop of glycerin; puts on a cover-glass, and warms gently for five minutes. The preparation is then washed with water, dried in the flame, and imbedded in balsam.

The results were even better when glycol or glycerin-ether were used instead of glycerin.

As attenuants of the staining solution and as decolorants of the tissue stained, the author used a large number of substances, but found that the best decolorizing results were obtained when physical and chemical extractives were used in combination, e.g. permanganate of potash and peroxide of hydrogen.

NASTUKOW, M. M., & M. J. PEWSNER-Ueber Sublimat-Anilin-Farbstoffe in der Bakteriologie. (On Sublimat-anilin-colouring Matters in Bacteriology.)

Wratsch, 1892, pp. 310-1 (Russian). SABOURAUD, R.-Quelques faits relatifs à la méthode de coloration de Lustgarten. (Some points regarding Lustgarten's Staining Method.)

Ann. Inst. Pasteur, 1892, pp. 184-340. UNNA, P. G.-Die Färbung der Mikroorganismen im Horngewebe. (The Colora-Hamburg, 1891, 8vo, 38 pp. tion of Micro-organisms in Horny Tissue.)

(5) Mounting, including Slides, Preservative Fluids, &c.

Shimer's new Mounting Medium.<sup>†</sup>—This is made of equal parts of Farrant's solution, glycerin, and glycerin-jelly, the last being made of gelatin, 30 parts; water, 70 parts; glycerin, 100 parts; carbolic acid, 2 parts. Of this jelly, liquefied by the aid of a water-bath, pour one fluid oz. into a 4-oz. glass stoppered bottle, add an equal volume of the Farrant's medium and of glycerin. Agitate, thoroughly mix, and add a small lump of camphor. A little warming is necessary to make it fluid for use.

The Short Slide as a Safety Slide. + Dr. Henry Shimer remarks :--"Much has been said in microscopical books, journals, and elsewhere,

\* Monatsschr. f. prakt. Dermat., xiii. (1891) pp. 225 and 286. See Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 315–7. † 'Pacific Record,' see Amer. Mon. Micr. Journ., xiii. (1892) p. 110.

<sup>‡</sup> Microscope, xi. (1891) pp. 266-70.

about care in using high power objectives, and warning of the danger of racking downward, &c. Having to use a fine, high power, dry objective of very short working distance, always nearly or quite touching the cover-glass when in focus, it is well known that the thickness of a series of cover-glasses of the same number varies greatly; hence, one of a package could be worked through, while another could not.

Looking across the stage and carefully racking down until the front of the lens was so close to the cover that I could not see between them with a hand-lens, thereupon applying the eye to the eye-piece, and manipulating the screw of the fine-adjustment, I often found that I was still above the focus, and it became an important consideration as to how close I could press the cover-glass with safety and advantage when searching for an object mounted in aqueous or glycerin medium. The little accidental motes when seen moving about in the medium sounded a note of alarm and said, 'You can go no closer; even now there is danger.' I have a so-called safety nose-piece in my possession, a contrivance with a spring in it between the tube and the objective, to prevent unbearable pressure, but it is not always on the stand; moreover, it is a troublesome thing in changing objectives where the stand has a short working distance, for it makes quite a long affair to handle and not touch the cover-glass in changing lenses after using a low power This safety spring admitted of an unpleasant pressure, as a finder. sometimes causing the mounting fluid to swell over the cover-glass, sometimes getting on to the lens, greatly to my annoyance. At this junction I looked about me for a better remedy than the safety nosepiece to use in my studies of objects on slides not finished by drying and sealing in a permanent mount. I then began to use the short slide as presently to be described. It was a new idea to me; whether new or not to other and more experienced workers, I cannot tell, but I do not remember having ever seen it mentioned in any of the microscopical books or journals at my command. I formed, in this way, a preference for slides 2 in. long and 7/8 in. wide, finally coming down to 1 in. and 3/4 in. long, and corresponding in length with the excellent short slides furnished by the Bausch & Lomb Optical Company, and very nearly corresponding with the length of the German slides furnished by Zeiss, of Jena, and to be had of Emmerick, in New York. Both of these, however, I found too wide for using as safety slides with my apparatus, the opening in the slide carrier on my No. 560 Bausch and Lomb stand being just an inch wide. I therefore was obliged to get the best, clearest glass I could find, and make my own slides, 7/8 and 3/4 in. wide by 1 and 3/4 in. long. A fine file or a piece of scythe whetstone rubbed over the edges and corners removes any sharpness, and is quickly done. A thousand slides of this kind can be easily and quickly made, and will answer every purpose in ordinary work. By mounting the object in one end I have a safety slide.

Place the slide so that the mounted end, when projecting over the opening in the stage, is free in air. Now it is apparent that when thus placed under the close-working objective it cannot be injured, because, when the point of the objective presses on the cover-glass ever so slightly, it can make no more than this slight pressure, for the slide, being placed see-saw-like over the opening, will begin the dip of the seesaw motion, and tell me that closer racking is useless. In this way I avoid the great care that is necessary in using the old 3-in. or long slide with the mount in the middle. This is without the least possible danger of injury to either the mount or the objective, while that is not. This is my first plea for the short slide. Such safety can hardly be furnished by any other device, and certainly no other is so free from annoying care. (2) The short slide is more conveniently stored in a horizontal till, which is preferable to sliding in the sawed grooves, and just as easily handled by the free end when we are accustomed to it. (3) The short slide is less likely to be broken if it accidently falls on a hard floor, the liability to break increasing as the square of the length, or more rapidly. A slide an inch long would hardly break once in a thousand falls, while one a foot long would most surely break at the first. (4) The short slide is lighter and more conveniently packed for transportation, which is important especially in the mails. (5) The short slide costs less, an item of some importance to many of us.

There are some objections to the short slide. The principal ones are as follows:—(1) It is not the standard slide. It is not the fashion, and to be out of fashion is a great load for many to bear. (2) The long slide may be better adapted to work on the turntable, which is a great convenience where a cell is to be made; still the circular cell can be made on the end of the slide with a suitable turntable. A square cell can readily and more quickly be made by hand, and a square cover can be cemented around the border more quickly than the slide can be placed on the turntable, and the square cover is better for most purposes, except for glycerin mounting, now nearly out of credit, and perhaps for dry mounting. (3) The long slide has some claims of moment in manipulating on the stage, in case we have no slide-carrier; but why be without a slide-carrier? If I had none I would at once improvise one with a thin piece of cigar-box lid, pasteboard, tin, or a long piece of glass, five or six inches long, the former, of course, with a hole cut in the middle. I have tried Microscopes formerly without slide-carriers, but after using one for fifteen years with a slide-carrier, I would almost as soon think of being without a Microscope as to be without this first and greatest convenience in manipulating the slide on the stage. If the opening in my slide-carrier were  $1\frac{1}{8}$  in. wide, as it ought to be, I would probably use all slides  $1\frac{3}{4}$  in. by 1 in., such as those sold by the Bausch & Lomb Optical Company, but, as it is, I have been obliged for some time past to work against another prejudice and use narrower slides, and I find a slide 3/4 or 7/8 in. wide ample for most uses, and for all covers 3/8 to 3/4 in. square, and is sufficient for the label; so what more do we want? If it is more labelling room, the two sides of the free end of the short slide can be used, thus affording plenty of space for the purpose.

The labels which I prefer for the purpose I cut out of gummed paper in slips 1/4 to 1/2 in. wide, and 2 to  $2\frac{1}{4}$  in. long as needed. Moisten and apply round the free end of the slide; after drying, write the name and number on the upper surface, and the mounting medium, date, and stain on the lower. In this way I find room for everything on the short slide.

I have heard it objected that the label under the slide places the latter out of level on the stage. This, at first sight, appears true, at least theoretically. But let us see how fine a theory it is. The thickness of a sheet of my gummed paper, measured by a micrometer, is about 1/1000 in., determined by holding the paper edgewise in a stage-forceps under the Microscope. In case we are using a power of 500 diameters, the field will be about 1/100 in. across. Now, by proportion, the length of the slide is to the diameter of the field as the thickness of the label is to the inclination of the field, or x. Then  $1\frac{3}{4}$  in., 1/100 in., 1/1000 in. x, 7/4 x = 1/100,000, x = 1/175,000 in., for the variation from a theoretical true level, or in the breadth of vision with a 1/5 or 1/6 objective; and if we are using a 2-in., 1/10 of that, which is 1/17,500, is out of level, on account of the level on the under side of one end.

Where is the instrument maker, however skilful, who can construct a stand the tubes of which shall vary so little as that from a true perpendicular to the stage? The best slides may vary more than that in the thickness of ends. Stands that raise one side of the stage by the fineadjustment, as do some of the Acme stands of J. W. Queen & Co., disregard this principle in a very much larger degree without detriment. This is my plea for the short slide. Will the reader allow me to submit it for its worth, without expecting to see its general adoption, but hoping to help some one who, like myself, is often bothered about the close working objectives, that in spite of all carefulness, occasionally impinge upon the cover-glass? Racking downwards is very troublesome to old eves using high powers. They find it difficult to look across the stage to reach below the focus, where the looking distance is 1/100 in. or less. Let all such try my safety slide, and they will find its perfect working convenience an ample compensation for being out of fashion or for lack of imaginary beauty, and after using it often and long, and trying it faithfully and well, they will, like myself, never wish to see another long slide for ordinary microscopical work."

# (6) Miscellaneous.

Squire's "Methods and Formulæ" used in Microscopical Examination.\*—The usefulness of this compilation will be obvious to any histologist, as it contains formulæ and advice for microscopical work in all branches of the subject save that of section-cutting. Great numbers of the formulæ are certainly correct, and the advantage of having these in a small and compendious volume will, no doubt, be evident to a great number of workers. For a first attempt the work is very excellent, but the formulæ for bacteria staining will require revision and amplification for a second edition. For example, no mention is made of the best and most rapid method of staining tubercle bacilli, the Neelsen-Glorieux, nor the most elegant and satisfactory, that of Czaplewski. It is satisfactory to note that no allusion is made to methyl-blue, a pigment which might easily be dispensed with, while with regard to methylen-blue, we would suggest the addition of a formula with an acidity corresponding to the alkalinity of Loeffler's methylen-blue.

Microscopical Examination of Potable Water.<sup>†</sup>-Mr. G. W. Rafter has just brought out a neat and practical little volume which deals with

- \* 'Methods and Formulæ,' J. and A. Churchill, 1892, 93 pp.
- † Van Nostrand's Science Series, New York, 1892, 18mo, 160 pp.

570
the microscopical examination of potable water. The author's aim is to make the biological method a check on the chemical analysis, for it is only by an accurate investigation of the number and character of living organisms that the exact value of free and albuminoid ammonia can be The present monograph only deals with non-bacterial estimated. organisms, and is divided into two parts, the first of which treats of methods, apparatus, &c., required for making a qualitative examination of a water supply. In the second part the subject is treated quantita-tively, the author showing how the organisms may be counted, and the inferences to be derived from their specific characters though "the sanitary significance and relative economic importance of the microscopical forms will be treated in another volume.

Filtration of Water through Stone Filters.\*-Prof. E. von Esmarch finds that the ordinary stone filters are, like charcoal filters, quite useless for sanitary purposes since they are only capable of removing the coarser impurities from turbid water. In one respect, indeed, they make matters worse, since they actually aid in propagating the poison they are pre-sumed to remove from the tainted water. The filters experimented with came from various parts, their filtering bed being composed of lava, tufa, or of sandstone. Naturally the great point to be ascertained was whether these filters would remove bacteria, and this was done by passing through Berlin water to which some pigment-forming bacteria had been previously added. It was found that the germs not only passed through, but after a certain lapse of time actually increased. It was obvious, therefore, that this increase must take place in the pores of the filter, a result which does not seem at all surprising since filters are not prone to possess a germicidal action, and it must be pointed out that the conditions of the experiment were unusually favourable for the development of microbes and quite different from those under which water is usually filtered. For example, a considerable quantity of organic matter, i. e. of the cultivation medium, must have been mixed with the water. All the same the experiments are worthy of record, as the use of filters no doubt leads to undue confidence in their virtue.

The Microscopic Structure of some Australian Rocks.<sup>†</sup>-The Rev. J. Milne Curran, in the concluding part of his paper, says :--- "A microscopic examination of our rocks points to the existence in Eastern Australia of every leading type from the vitreous to the holo-crystalline condition, both acidic and basic, and their general microscopic structure conforms to well-known types of described American and European In writing of American basalts, Zirkel says, t 'It is worth rocks. while to pause, and remark that in these widely remote quarters of the globe, the product of the solidification of a molten mass, although exposed to many casualties, has, nevertheless, maintained a surprisingly close identity of microscopical composition.' The remark applies in every particular to the Australian basalts described in this paper.

Within well-defined limits, the structure of our basalts shows micro-

<sup>\*</sup> Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 525-31.

<sup>†</sup> Journ. and Proc. Roy. Soc. N.S. Wales, xxv. (1891) pp. 179-233, pls. xx.-xxii. ‡ U.S. Geol. Explor. Fortieth Parallel, vol. vi. Microscopical Petrography,

p. 253.

scopical peculiarities that enable us to recognize certain types of structure as characteristic of particular districts, for instance, a microslice of basalt from Orange can always be distinguished from similar rocks at Bathurst.

In regard to the order of solidification of minerals, as a rule nothing exceptional is to be noted in the material examined. The cavities of some basalts are filled, as at Carcoar, with aragonite. There is nothing in the microscopic character of the slices to show that the lime for this mineral was derived from the surrounding rock. On the contrary, there are Tertiary volcanic rocks at Rocky Bridge Creek with their cavities filled by opal and hyalite, and the Microscope shows that this material has been derived from the silicates of the rock itself.

As far as it is yet known the great bulk of the eruptive rocks of New South Wales are basic in composition. Intermediate rocks are not common. Acidic rocks are rare. There are extensive metalliferous deposits about Cobar, but the Microscope reveals no intrusive rocks in connection with these deposits.

Many of our granites are suffering from a decay called by Dolomieu 'la maladie du granit.'\* Carbonic acid gas in the air, as has been suggested, may have a good deal to do with this process of disintegration, but the Microscope shows that to a certain extent the disease is internal. The quartz of some granites is seen to contain numerous gas cavities, and the cloudiness and incipient kaolinization of the felspars is probably due to the absorption of the free gas they once held.

Many of the rocks called diorites are augitic rather than hornblendic, and therefore must be classed with diabase. In the conversion of a clay-slate to a hornfels, as at Bathurst, the Microscope shows that the alteration of the rock consists in a rearrangement of the old minerals, and the introduction of one new one, namely mica. This corresponds with observations made on similar rocks in other parts of the world. The wide distribution of Tertiary leucite rocks in New South Wales is a matter of considerable interest.

The specimens with which this paper deals have been collected over widely separated localities. There is material enough in any one district for considerable petrological research, but it has been my purpose rather to indicate the wealth and variety of our material for work, than to give exhaustive details of any one field. Much of the matter furnished will, I trust, prove new and of some interest to Australian geologists."

\* Lyell's 'Principles of Geology,' 11th edition, vol. i. p. 409.

572

( 10 )	
DR. HENRI VAN HEURCK'S M	IGROSCOPE
FOR HIGH-POWER WORK AND	
PHOTOMICKOGRAPH	.1,
AS MADE BY W. N THE SPECIFIC/ HEURCK C	WATSON & SONS TO ATION OF Dr. VAN DF ANTWERP.
Fitted with Fine A sensitiveness an to derangement	Adjustments of utmost ad precision, not liable by wear.
Has Rackwork Draw tives to the thic Can be used with	w-tube to adjust Objec- kness of Cover Glass. either Continental or
English Objectiv Fine adjustment to The Stand speciall utmost convenie	ves. Substage. y designed to give the ence for manipulation.
As Figured, with 1	Eyepiece £18 10s.
Also made with Cor	finental form
Without Rackwork	to Draw-tube £16
	Full description of the
	above instrument, and
	Microscopes, and appa-
	list of 40,000 Micro-
	warded post free on application to
	W. Watson
	&
	Sons,
	313 High Holborn,
	LONDON, W.C.
Artic manth	AND AT
	78 Swanston Street,
	Melbourne,
	Australia.
	ESTAP.
	1097

Awarded 28 GOLD and other Medals at the principal International Exhibitions of the World.

-1-

HILLING BURNER IN

## MICROSCOPY.

## a. Instruments, Accessories, &c.\*

Guide to Microscopical Technique.<sup>†</sup> — This The Microscope. small text-book of 140 pages contains a concise though fairly complete account of (1) the Microscope and its accessories; (2) the use of the Microscope; (3) preparations; (4) graphical representation of preparations. In the first section is a short history of the Microscope, followed by a description of its optical arrangements, from those of the very simplest to those of the most perfect instruments replete with the latest improvements. The second section treats of the use of the instrument, and contains a full account of the methods of adjustment and illumination, the different sources of light, &c. The third section deals with the production of preparations of various materials. It also contains directions for the treatment of living objects, and for the cutting of sections, &c. The last chapter is devoted to the graphical representation The various kinds of drawing apparatus are first of preparations. described, and then the methods and appliances for photomicrography and projection are fully discussed. The whole book is plentifully illustrated with plates and figures.

#### (1.) Stands.

Zentmayer's American Continental Stand.—This stand has been designed to meet the wants of those workers who while preferring the compact Continental model are conscious of its inherent defects. It is substantially a combination of the upper half of American with the lower half of the best Continental stands.

The stand is constructed entirely of brass; the base is of horse-shoe form, filled with lead for extra weight, and gives perfect steadiness in every position. A stout pillar firmly supports the arm of the instrument on a trunnion-joint, which allows all inclinations from the perpendicular to the horizontal position. The coarse- and fine-adjustments are of the same style and construction as in the Centennial stand. The arm carrying the body is provided with two slides, the upper and longer one bearing the tube with rack-and-pinion movement, and sliding in the lower one, which is controlled by a lever of the second order, operated by a milled-headed micrometer screw in convenient position at the back of the instrument. At the bottom of the lower slide there is a shoulder against which the lever acts, and a spring above presses down against this shoulder, insuring its continuous contact with the lever during adjustments. All the mechanism is concealed within the arm, which is so hollowed as to secure both lightness and greater rigidity. This fineadjustment is absolutely free from lateral motion, and exceedingly sensitive. Its construction prevents wear, and a revolving nose-piece and attached objectives can be easily carried without injury. It also acts as a safety appliance in case an objective is accidentally racked down against an object, for the spring yields quickly to upward pressure. The body-tube is  $5\frac{1}{2}$  in. long, with draw-tube extending full 10 in., thus

\* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous.

† Schweiger-Lerchenfeld, Λ. v., 'Das Mikroskop. Leitfaden der mikroskopischen Technik nach dem heutigen Stande der theoretischen und practischen Erfahrungen.' Wien, Pest, and Leipzig, 1892, 8vo, 192 figs. See Bot. Centralbl., l. (1892) pp. 261-2. giving both English and Continental standards, and accommodating objectives corrected for either length. The spacious stage is made of aluminium, which is incorrodible; the dimensions  $(3\frac{5}{8}$  in. square) are commodious even for culture slides or serial sections; the surface is plane, with recessed opening to receive a glass plate, light-modifier, or disc-



diaphragm, if wanted; removable clips are provided, with springs shaped and adjusted to hold a slide, and yet allow easy movement about the field of view. The substage has long sliding movements in the fixed bar beneath the stage, allowing ample room for a condenser or polarizer, and exact adjustments are easily and quickly made by aid of milled knobs extending on each side of the sliding bracket, on which the ring of the substage is centered and affixed instantly by means of a single set-screw with capstan head. The mirrors are plane and concave, of large size, and have complete adjustments on an extensible bar. The diaphragms are cone-shaped, and have three different sizes of The Abbe illumiapertures. nating apparatus has a condenser of 1.20 N.A. and iris diaphragm with complete movements. Α condenser of 1.40 N.A. can be substituted, if preferred. A set of stops are also furnished for dark-ground illumination.

A modification of the swinging substage and mirrors has been effected, whereby the extensible mirror-bar slides in another bar which swings from a joint on the under side of the swinging-

bar carrying the substage. This construction allows the substage and mirrors to swing independently of each other, click-stops indicating when either or both bars are in the optic axis of the instrument; and permits the substage to be swung aside entirely and the mirrors alone to be then swung into positions for central or oblique illumination, without interference from the substage. The mirrors can be likewise swung aside completely to permit the use of direct illumination, with or without substage apparatus. These movements contribute much to convenient and rapid use, as it is unnecessary to remove and afterward return the substage, or mirrors, or any other part. In this instance the stage is made somewhat narrower to allow the substage to swing clear aside. Fuess Microscopes.\*—The Fuess model No. II. (fig. 68) is similar to the larger instrument described in this Journal, 1891, p. 393. The stand can be inclined to the horizontal. The rotating stage is divided



into  $360^{\circ}$ , with two verniers reading to 5 minutes. It can be fixed in any position by means of a lever. The polarizer has a rack-and-

\* Fuess, R., 'Krystallographische u. Physikalische Instrumente,' Berlin, 1891, 56 pp., 55 figs.

# 666 SUMMARY OF CURRENT RESEARCHES RELATING TO

pinion motion. In its socket fit the various illuminating apparatus, which can be centered by slide pieces provided with screws. Arrangements for obtaining convergent and parallel light can be inserted.



The mechanical stage (fig. 72, p. 669) is similar to that of the larger model, but has no micrometer measuring arrangement.

All the special eye-pieces and other accessories of the model No. I. can be used with this instrument.

Model No. III. (fig. 69) does not differ very much from the preceding. The stage is provided with cross-divisions for the orientation of the preparation; but if required the mechanical stage of model No. II.



can also be applied to this instrument. The draw-tube is only movable by hand, and carries no millimetre divisions.

In model No. IV. (fig. 70) the stage is fixed. The only focal adjustment is by rack and pinion, which, however, is sufficiently fine for the use of objective No. 7. The instrument is provided, like the others, with means for fixing the stage, polarizer and analysers, arrangements for convergent and parallel light, Bertrand lens, objective clamp, &c.



Model No. VI. (fig. 71) is in size and construction precisely similar to model No. II., but differs from it and the other instruments by a special arrangement for the simultaneous rotation of the two nicols. This construction is obviously borrowed from the Dick Microscope as made by Mr. Swift, and described in this Journal, 1889, p. 432. The



Microscope is particularly suitable for the observation of glowing minerals in polarized light.

Combination of Microscope and Reflecting Goniometer.\*—Prof. A. Schrauf describes a combination of Microscope and goniometer which is useful for the measurement of minute crystals; the ordinary telescope of the goniometer fails to give sufficient magnifying and defining power to enable the edges of very small crystals to be properly centered and adjusted. For the measurement of such crystals he proposes to add to the ordinary instrument a Microscope directed vertically with its line of sight passing through the axis of the goniometer. Since the collimator and telescope are inclined at  $35^{\circ}$  to the horizontal, the Microscope can be easily supported by a side tube vertically above the crystal. The ordinary telescope serves for approximately centering and adjusting the crystal, while the more exact adjustment is made with the Microscope. According to the intensity of light from the faces of the crystal, the following methods of observation may be applied.

(1) The complete Microscope is used. In the goniometrical measurements the faces belonging to one zone will then appear successively in the field of view. By arresting them at the maximum of their intensity "Schimmer" measurements are obtained.

(2) By separating the whole Campani eye-piece, an image of the signal, as formed by the objective, is seen and can be used for approximate measurements.

(3) If the upper lens only of the Campani eye-piece be removed, a double appearance is seen on the reflecting face of the crystal. The face no longer shines with a uniform light, but gives a series of clear signals close to one another. The "Signal-Schimmer" measurements obtained by this means are more correct than those of (1).

\* Zeitschr. f. Wiss. Mikr., ix. (1892) pp. 128-30.

(4) By inserting between eye and body-tube a Ramsden eye-piece, a single clear image of the signal, which can be adjusted on the cross wires, is obtained.

(5) The above methods are equally applicable to the simple goniometer without telescope and collimator.

## (2) Eye-pieces and Objectives.

Fluorite in Apochromatic Objectives.\* — Mr. E. M. Nelson remarks — "As fluorite is becoming scarce, an important question arises as to whether fluorite is or is not present in any given lens. This can readily be determined by means of a polariscope.

The Nicols are crossed, a 2-in. objective is placed on the nose-piece, and a low eye-piece employed. The various portions of the objective to be tested are unscrewed, and each combination is separately placed on a glass slip on the stage and examined in the dark polarized field. If the combination contains fluorite there will be a luminous white silky appearance, but if there is no fluorite, then the field will remain dark.

The following are examples :— The Zeiss 24-mm. apochromatic has three elements, of which the middle contains fluorite. The apochromatic 12 mm. has four elements, of which the second and back contain fluorite. The apochromatic 6 mm. has three elements, of which the middle and back contain fluorite. The apochromatic 3 mm. has five elements, and the last but one contains fluorite.

But with regard to this last example, it should be noted that all 3- and 2-mm. objectives are not alike."

# (3) Illuminating and other Apparatus.

A New Spherometer.—Mr. E. M. Nelson communicates the following:—Nearly all spherometers are graduated in dioptries, as they are chiefly employed for measuring the foci of spectacles. This graduation is inconvenient if you require the radius only. The size of the spherometer ought to bear some proportion to the size of the lens to be measured, consequently for general purposes more than one spherometer would be required. A large lens, for instance, with shallow curves, can be more accurately measured by a large spherometer, which would not measure a small lens at all; moreover, as spherometers are expensive instruments, and as at least three of them would be required, it seemed better to design one that would be suitable, and, at the same time, be as simple in construction and as accurate as any hitherto made.

The usual form of spherometer is that shown on p. 131, fig. 19, of this Journal for last February, in the admirable paper by Prof. Silvanus Thompson on his new focometer.

The principle on which these instruments depend is the measurement of the versed sine with a given chord.

Now the defect in the principle employed in the spherometer shown in fig. 20 is that you cannot be sure that the chord between the two fixed points is that of a great circle of the sphere; if it is not, the result will be inaccurate.

\* Journ. Quek. Micr. Club, v. (1892) p. 122.

By the three fixed points in fig. 19 that error is eliminated. But to make three points which shall include accurately a given chord is a matter of some difficulty; I therefore determined to simplify mine by



F1G. 73.

A, lens being measured; B, ring; C, drum-head; D D, legs of tripod, 9 in. long; E, glass plane surface; F, lens for examination of Newton's rings.

dispensing with the three points altogether, and by substituting for them a ring. The ring is of course much cheaper to make, and that, too, with less liability to error.

The screw is common to both, and the error on that score is the same in either case; but with regard to accurate centering the ring probably has rather an advantage.

There is a difference in the way of using them, for while the spherometer in fig. 19 rests on the lens, in this one the lens rests on the spherometer.

My spherometer consists of a cylindrical ring supported on a tripod ;

in the centre of this cylinder is a micrometric screw of 50 threads to the inch, having its lower end fixed to the drum-head. The edge of the drum-head is divided in the usual way, each division representing 1/10,000th inch. The upper row of figures are for measuring convex lenses, while the lower are for concaves.

The upper end of the screw which touches the lens terminates in a small polished hemisphere of steel, contact being indicated by the formation of Newton's rings which can be seen through the lens which is being measured.

Only the lower edge of the ring is in contact with the inside of the drum-head, and contact at this point steadies the screw.

The figure shows the instrument in use with a full-sized ring; but when a small lens is to be measured the full-sized ring is replaced by a smaller one, the same screw and drum-head being used in all cases.

This instrument has three rings, and is practically three spherometers.

Before using the instrument a glass disc 1/2 in. thick, worked on its under side to a true plane surface, and having a plano-convex lens cemented to its upper side, is placed on the ring; the drum-head is then turned until the hemispherical end of the screw comes in contact with the lower side of the glass disc; the Newton's rings formed by this contact will be plainly seen through the lens on the upper side of the glass disc. When this contact is made the index on the drum-head should be at zero; if it is not it can be placed there by means of the adjusting screws below the drum-head.

With regard to the diameters of the rings, if a chord of suitable dimensions is chosen the arithmetical computation of the radius can be much shortened. Thus—

Let R be the radius, C the chord, and V the versed sine which is measured, then

$$\mathbf{R} = \frac{\mathbf{V}^2 + \left(\frac{\mathbf{C}}{2}\right)^2}{2 \mathbf{V}}.$$

If, therefore, we make  $C = \sqrt{8} = 2 \cdot 82843$  inch,

$$\mathbf{R} = \frac{\mathbf{V}}{2} + \frac{1}{\mathbf{V}} \cdot$$

Or  $C = \sqrt{2} = 1.41421$  inch,

$$\mathbf{R} = \frac{1}{4} \left( 2 \mathbf{V} + \frac{1}{\mathbf{V}} \right).$$

And if  $C = \sqrt{\cdot 2} = \cdot 447214$  inch,

$$\mathbf{R} = \frac{1}{40} \left( 20 \, \mathrm{V} + \frac{1}{\mathrm{V}} \right).$$

As  $\frac{1}{V}$  can be taken out of a table of reciprocals, the computation becomes one of mere inspection.

The above are the diameters of the three rings of this instrument.

672

#### (4) Photomicrography.

Photomicrographical Apparatus.\*— Prof. Martens has fitted up in the Königliche Mechanisch-Technische Versuchsanstalt in Berlin a photomicrographical laboratory which is almost unique in the completeness of the arrangements for the illumination of opaque objects.

The Microscope employed was made by the firm of Zeiss after-Prof. Martens' design. It stands on a slide movable in the direction of the optic axis, and is fixed to a cast-iron plate which rests on two fixed points and an adjusting screw, so that the optic axis of the Microscope can be suitably directed. Both the body-tube (50 mm. wide), and the movable stage, which is provided with a coarse- and fine-adjustment, are directly attached to the common support The extent of movement of the stage is considerable, in order to facilitate the examination of large The stage is of the same form as that in Zeiss' photographic objects. instrument. By means of the usual spring clamp an object-holder can be attached to it, so that in the case of irregularly shaped objects the surface to be examined can be properly adjusted. For the illumination by reflected light several methods are adopted. For weak objectives either a plane parallel glass set at 45° in front of the objective is used, or a prism placed so far forward as to cover one-half of the aperture of the objective. Both arrangements are carried by an adjustable holder attached to the body-tube. For higher objectives illumination from above through the objective is employed. For this purpose the light from a side opening is directed by a prism through one half of the objective so as to fall upon the object which is examined by the other half of the objective. In this arrangement the Zeiss apochromatics 0.30 and 0.95 N.A. (16 mm. and 4 mm. focal length) are used. The prism-holder consists of a small box which can be displaced sideways so as to give the effect of oblique light, or be shifted quite to one side, when the objective can be used with its full aperture for illumination by transmitted light. For the adjustment of the prism at right angles to the optic axis of the illuminating apparatus, a piece is provided which is movable about the optic axis and carries both objectives. This holder with the apochromatics and special prism can be replaced by another with fixed prism into which Zeiss objectives A, B, and D D fit.

Photomicrographical Apparatus of the Leipzig Anatomical School.<sup>†</sup>—Prof. W. His describes the photomicrographical apparatus which he employs in his embryological and morphological researches. The apparatus is not intended for the more difficult problems of photomicrographical technique, but to answer the more modest requirements demanded by work at comparatively low magnifications. The aim of the author was to produce, under moderate magnification (10 to 200 times), large pictures which could be of service for precise measurements and plastic reconstructions. The work was simplified by dispensing with the glass negative and taking the photographs directly on Eastmann's silver bromide paper. The apparatus first used was arranged

† His, W., 'Der Mikrophotographische Apparat der Leipziger Anatomie' (Festschr. Albert Kölliker zum 26. März 1892), Leipzig, 1892, 22 ρp., 3 pls. and 2 woodcuts.

1892.

<sup>\*</sup> Central-Ztg. f. Optik u. Mechanik, xiii. (1892) pp. 135-6.

# 674 SUMMARY OF CURRENT RESEARCHES RELATING TO

for gaslight, and only admitted of magnifications up to 20 times. The present apparatus, in which the electric light is employed, allows of work at any magnification, from the lowest to the highest attainable with immersion apochromatics.

The general arrangement of the apparatus is seen in the figure (fig. 74) which represents the ground plan of the photographic room. T is the work-table, I the lantern with the electric lamp L. B the first illuminating lens, II the optical bank with slides 1 to 3, K a zinc vessel with water supply for cooling the lantern, III the stand in the dark room, R the photographic frame.



FIG. 74.

The electric lantern consists of a wooden box 95 cm. high, containing a Bürgin arc lamp. In the side wall is a window for the observation of the arc, and in the front wall fits a metal plate, which is movable transversely by screws, and carries a wide tube containing the first illuminating lens. Between lamp and lens is a copper cooling vessel, with glass sides, through which a stream of cold water passes. For centering purposes the lamp can be displaced transversely on a slide by means of a winch worked from the outside. Each of the two carbons is provided with an independent movement. The most advantageous distance for the carbons was found to be 4-8 mm.

The optical bank, 61 cm. long, is provided with prismatic rails, and rests on two iron supports built into the wall of the room. Three slides move on it, the first of which carries the illuminating system, the second the object-stage, and the third the objective. The illuminating system consists of a double lens of 21 cm. focal length. Besides this lens, slide 1 also carries a red glass plate which can be thrown out to one side, and a metal ring for the reception of coloured glass plates.

Slide 2 supports a brass frame on which the object-stage hangs. This stage, which is movable by screws, horizontally and vertically, can be readily put on or off.

Slide 3 carries a rectangular metal plate in the ring of which the objective is screwed. For high magnifications this objective-holder can be removed and replaced by a large Zeiss Microscope. In front of the objective is a diaphragm which can be thrown out to one side. Slides 1 and 2 are adjustable by hand. Slide 3, on the other hand, is moved by a screw which can be worked from the interior of the dark room.

The front wall of the dark room, 10.5 cm. from the optical bank, carries the projection aperture with its shutter, besides a loop-hole and a small velvet curtain for protection against reflections. The projection aperture measures 8 cm. in diameter; the shutter is a plate of sheet iron movable about an axis. This plate is covered on its outer surface with white paper, and thus serves for the proper adjustment of the light, for it is easy to recognize on the plate whether the light is evenly distributed or whether coloured edges, &c., are present. The space between objective and projection aperture is covered by a velvet cloth.

The photographic frame (fig. 75) is moved by a winch along two racks on a support,  $2 \cdot 1$  m. long and  $0 \cdot 95$  broad, fastened to a fixed stand in the dark room. For the author's purpose of obtaining large photographs directly on paper the following arrangement was employed. Two thick glass plates, 80 cm. square, are fitted into two wooden frames in such a way that they can be brought almost into immediate contact, so that a sheet of paper can be held firmly between them. The two frames are fastened together by two screws with bayonet catch. The vertical position in the main frame is marked by a stop. The hinder glass plate is covered in front with white paper on which the adjustment is made.



For the purpose of reducing the intensity of the electric light the author uses coloured glasses, and almost exclusively dark yellow ones.

The times of exposure in the author's experiments varied from 10 seconds as a minimum to three minutes as a maximum, but were mostly from 30-60 seconds. For magnifications up to 200 or 300 the objective alone was sufficient, for higher magnifications the large Microscope-stand and projection eye-piece of Zeiss were used.

As regards the choice of the systems, the author, from the experience of several years, considers that for low magnifications the Steinheil aplanatics leave nothing to be desired. He uses for magnifications of 4-15

2 z 2

times the aplanatic of 14 cm., for 8-25 times that of 7 cm. focal length. Many of the Hartnack systems, however, are not inferior to these. For magnifications of 20-55 Hartnack's embryograph was found to be the best. For medium magnifications of 50-300 times the Seibert systems of 1, 1/2 and 1/4 in. were used, and for magnifications above 300, the Zeiss projection eye-piece with the dry apochromatics of 8 and 4 mm. focal length.

With high magnifications a difficulty was experienced with respect to the clamping of the preparation on the object-stage. Owing to the unequal pressure of the two clamps the preparation was not at right angles to the axis of the Microscope. This difficulty was removed by attaching to the stage a plate which could be levelled by screws. The clamps, three in number, were screwed to this plate, and their pressure on the object-slide was thus regulated.

The course of the operations for low or medium magnifications is as follows. After fixing the objective and closing the current in the electric lamp, the magnification is determined. For this purpose a small glass scale divided in half or tenth millimetres is adjusted on the stage of slide 2, and the frame in the dark room is moved until the desired magnification is obtained. The proper illumination is then considered. The first illuminating lens (20 cm. focal length) in the lantern is once for all adjusted so that the rays fall approximately parallel and fill the aperture of the second lens on slide 1.

This slide is then displaced until the pcint of the pencil of rays falls in the plane of the diaphragm of the objective.

The preparation is now placed on the stage, and the fine-adjustment is made from the dark room. The current is then broken and the two shutters and the front door of the dark room are closed.

The plate-frames are then laid down and the sensitive paper inserted between them. The current is once more closel and the light regulated by examination of the white paper in front of the shutter. To ascertain whether the image falls properly on the sensitive paper the red glass screen is placed before the illuminating lens of slide 1, and the shutter is opened, while the object-stage is adjusted. The shutter is then closed again, the red glass removed, and the light again adjusted. Exposure is then effected by opening the shutter.

To adjust the apparatus for use with the Microscope-stand, the objective-holder is removed and space cleared for the stand. The Fritsch's wheel on the slide is then connected by an intermediate piece with the guide in the dark room, and the diaphragm-holder of slide 3 is adjusted. The Microscope is now screwed upon the slide and directed horizontally. The Fritsch's wheel is raised until it engages in the micrometer screw of the Microscope. To insure the proper centering of the light the aperture of the iris-diaphragm is made very small, and the illuminating lens and carbons are moved until the point of the pencil of rays falls just in this aperture. For the later orientation on the direction of the pencil of rays a second diaphragm in the diaphragm holder is used. The circle of light is made concentric with the aperture of the diaphragm.

After the centering of the light, the iris diaphragm is opened, and by means of the large illuminating lens and Abbe condenser the proper illumination of the object is determined. The other operations are as before.

Of the three excellent plates in photogravure at the end of the book, prepared by Riffarth of Berlin and Albert of Munich, the first gives a perspective view of the optical bank and its connection with the lantern and front wall of the dark room. The second and third are photographs of sections taken on Eastmann paper. Plate II. is the frontal section through head and neck of a human factus of the fourth month ( $\times$  9). Plate III. is a section through the spinal column of a four weeks' human embryo ( $\times$  210).

Use of Photography in Natural Science.\*-Prof. G. Marktanner-Turneretscher discourses on the various ways in which photography can assist in scientific work. He first takes the case of museums and scientific institutions, and points out how useful for the zoologist photographs of rare and type specimens would be. More especially serviceable would be photomicrograms of minute specimens. Whole groups, such as the Protozca, which are too small for exhibition, could thus be brought to the notice of the public. Such photomicrograms would be also of great assistance to the scientist himself, since they can be compared with other specimens &c., without the necessity of bringing the object itself again under the Microscope. If each scientific worker had photographs of his species arranged as a catalogue the task of comparison of new with already known forms would be considerably simplified, and many synonyms would in this way be avoided.  $\mathbf{As}$ regards the palaeontological departments of a museum, the less perfect specimens should be accompanied by photographs of the more perfect individuals which had been figured.

The value of photography and more especially of photomicrography to the histologist and embryologist is seen in the constant use which they make of it, as shown by the numerous photographic plates which invariably illustrate their published works.

In botanical researches photography has also been of the utmost service, as, for example, in the recent investigations of Paul Knutt on the fertilization of flowers by insects. He found that certain flowers with little colour produced a considerable effect on the sensitive plate. He explains this as due to the reflected ultra-violet rays, and considers, from the fact that such flowers are sought after by insects, that the insect eye is sensitive to these rays.

In mineralogy and petrology photomicrograms of sections in ordinary and polarized light are of great use. Each rock specimen exhibited in a museum should be accompanied by a photomicrographical representation of its section.

In teaching establishments photography is more particularly of service by the methods of projection.

The author concludes with advice as to the most suitable photographic apparatus to be used for different purposes. As regards photomicrography he recommends the use of Zeiss' apochromatics and projection eye-pieces for work with high magnifications.

\* Mittheilungen d Section f. Naturkunde des O. T.-C., iv. (1892) pp. 33-5 and 41-44.

## 8 SUMMARY OF CURRENT RESEARCHES RELATING TO

## (5) Microscopical Optics and Manipulation.

Abbe's Method and Apparatus for the Determination of Focal Lengths.\*—Dr. S. Czapski gives a detailed account of the methods and apparatus recently devised by Prof. Abbe for the determination of focal lengths,† and explains the principles on which they depend. By the Abbe method, the fundamental conditions necessary for "micrometric measurements by means of optic images" are more fully satisfied than by any other previously employed; and accordingly the influence of the systematic as well as the accidental errors of observation on the result are considerably minimized. These fundamental conditions, as formulated by Prof. Abbe, are as follows :—

(1) A determination of precision must not be made dependent upon the position of the optic image.

For this position is always uncertain. The amount of play in an adjustment is directly proportional to the keenness of the observing eye and inversely to the angular aperture of the pencil forming the last image in front of the eye. For a normal eye the amount of play amounts to about 13 mm. For an eye armed with a lens this source of error is considerably increased. In the determination of the focal length of optical systems therefore, the distance of one image from another or from a fixed point must not enter in as a factor.

(2) The measurement must not be indirectly affected by the adjustment.

Suppose, for example, that the magnitude of the image P Q = y' formed by the system S of the object O M is to be measured, and that



an error in adjustment Q Q' = d x' has been made; then the amount of error in the measurement of P Q is R P' = Q Q' tan P S Q, i.e.  $d y' = d x' \tan \omega$ .

The error involved in calculating the magnitude of the object from that of the image is then

$$\frac{d y}{y} = \frac{d x' \tan \omega}{N y}$$

where N = y' / y is the linear magnification.

A means of reducing the error is to diminish the angle P S Q, which the axis of the pencil forming the image makes with the axis of the

\* Zeitschr. f. Instrumentenk., xii. (1892) pp. 185-97. † See ante, p. 427.

678

#### FIG. 76.

system. Prof. Abbe makes the angle zero by the use of a sufficiently small diaphragm in the front focal plane of the system, the effect of which is to make the system on the side of the image "telecentrisch," i. e. the axes of all the pencils emerging from the system, as seen in fig. 77, are parallel to the axis of the system.

(3) The third requirement in any method of precision depends on the following considerations :—

It is in the nature of dioptric systems that in general the images are not in all parts proportional to the objects, but that the ratio of the



magnitudes of the two is a function of their dimensions. For this reason, if conclusions on the fundamental properties, e. g. the focal length, are to be drawn from the magnification which a system has in any two conjugate points, only a very small central part of the image must be used in the measurement. On the other hand, however, the measurement of the magnification is always more exact, the greater the object and image.

The simplest way to satisfy these somewhat conflicting requirements is to take several measurements on large images. gradually diminishing in size, and from the result of these relatively exact measurements, to calculate the fundamental value of the magnification, i. e. that value which would be found for the infinitely small central part of the image. The author shows how these three requirements are fully satisfied in the Abbe method for determining focal lengths.

In this method, the determination of the focal lengths depends upon the magnifications which the system gives of two objects at a determined distance apart.

If f is the focal length of the system,  $N_1$  the magnification for one pair of conjugate points,  $N_2$  that for another pair, and a the distance of the object planes apart, then (fig. 78)

$$f = \frac{a}{\frac{1}{N_1} - \frac{1}{N_2}}.$$

Two exactly divided scales in  $O_1$  and  $O_2$  serve as objects. The first requirement is satisfied by the fact that it is the distance of the objects and not that of the images which has to be measured.

In order to satisfy the second, a small diaphragm may be brought into the front focal plane F of the system, and measuring arrangements, for determining the magnitude of the images, in the planes  $P_1$  and  $P_2$ , behind the system.

# 680 SUMMARY OF CURRENT RESEARCHES RELATING TO

None of the measuring arrangements, however, at present known would sufficiently satisfy the third requirement by presenting a large enough part of the image for measurement. To obviate this difficulty a micrometer-Microscope may be made to move parallel to itself, and to the axis of the system from one end of the image to the other, e. g. from the ray E to the ray G. Different parts of the image are thus brought



into the field of view, and the magnitude of the displacement required in order to bring certain parts of the image into a determined part of the field, is equal to the distance between those parts of the image. When the micrometer-Microscope is provided with cross-wires in the eye-piece, a further simplification follows. For if the axis, i.e. the line joining the centre of the cross wires to the hinder principal focus of the micrometer-objective, is parallel to that of the system to be measured, and keeps parallel to it during the displacement at right angles to that axis, then only pencils, the axes of which are parallel to that axis, come to a focus. Thus by this arrangement the telecentric path of the rays is obtained without the necessity of inserting a diaphragm in the front focal point of the system.

An arrangement however to effect the exact parallel displacement of the Microscope, if technically possible, would render the instrument costly and perhaps detract from its functional exactness. To avoid this, Prof. Abbe, instead of making the measuring arrangement (the Microscope) displaceable with respect to the objective, has made the latter movable at right angles to its axis and parallel to itself, while the Microscope and scale remain fixed. The displacement Y of the objective necessary to pass from the image of one point of the object to another is then the magnitude of the image; the magnitude of the object to be compared with this is equal to this displacement, minus the real distance y of the two points of the object which were successively adjusted, i. e. to Y - y. If then  $\beta_1$  and  $\beta_2$  denote the reciprocals of the magnifications  $N_1$  and  $N_{22}$  we have

$$f = \frac{a}{\beta_1 - \beta_2}$$
 where  $\beta_1 = \frac{Y_1 - y_1}{Y_1}, \ \beta_2 = \frac{Y_2 - y_2}{Y_2}$ 

The apparatus itself (figs. 79 and 80) is essentially a large Microscope. Almost the only difference is that in the lower part there is a metal frame for the reception of a glass scale T. A second more finally





divided scale t is fitted just beneath the stage, and can be moved backwards and forwards by a small lever H. Its central position is marked by a stop.

Dovetailed in the stage of the Microscope is a plate W, movable from right to left, and carrying on the edge nearest the observer a division on silver s with a vernier N reading to 0.05 mm.

The glass scale T is divided in 1/2 mm.; the scale t, in the central portion of 2 mm. length, in 0.05 mm.

The body-tube has the usual coarse- and fine-adjustments.

The draw-tube carries a millimetre division, giving the total tubelength. For use with the apparatus there are five objectives, numbered 1-5, and an eye-piece, with removable optic lens, in the diaphragm of which either a double cross wire or a micrometer scale (10 mm. divided in 0.1 mm.) can be fitted. The objectives are screwed to the body-tube, and centered by means of the arrangement movable by the screw Z. The eye-piece simply slides into the body-tube, and is clamped in position by a screw.

The height of the tube-support above the stage is 50 mm., so that measurement can be made of systems of this height, and with a diameter of 100 mm.

As at present constructed, the apparatus serves for the measurement of systems of somewhat more than 80 mm. focal length, and of about 20-100 mm. aperture.

In making an observation with the instrument, the objective 1 (of shortest focal length) is first used, and the cross wire in the eye-piece is arranged with the double wire from front to back. The Microscope is then adjusted on the scale t, which is illuminated by the mirror beneath the stage. The objective is centered so that the middle division of the scale appears between the double wire in the eye-piece, and the position is noted in which the horizontal cross wire cuts the micrometer division. The movable plate of the stage is then displaced until the zero of the vernier points to the middle division of the scale s.

The system S to be measured is now placed on the scale t, and is moved, while the Microscope is again adjusted on the scale, until the same mark of the scale t appears between the double wire of the eye-piece, and the horizontal wire cuts the image of the division of t at the same height as before.

The plate W is now moved from the central position to the right until a certain division of the scale t appears between the double wires of the eye-piece, and the Microscope is readjusted on the scale. A reading is then taken on the vernier of the scale s, and the distance of the division of t from the central division noted.

The plate W is now displaced to the opposite side of the central position, to the left, until the corresponding division of the scale as before, only on the other side of the middle division, is adjusted in the eye-piece. The displacement of the scale s is again noted.

The same processes are then repeated for the scale T, which is rendered visible by the removal of the mirror and the upper scale. The objective 1 is in this case replaced by one of the lower ones chosen by trial.

Let  $y_1$  and  $y_2$  denote, for the two scales respectively, the distance of

682

the two divisions adjusted by the movement from right to left, and  $Y_1$ ,  $Y_2$ the displacements of the scale s, then, since

$$f = \frac{a}{\beta_1 - \beta_2} \text{ where } \beta_1 = \frac{Y_1 - y_1}{Y_1}, \ \beta_2 = \frac{Y_2 - y_2}{Y_2},$$
$$f = \frac{a}{y_2 - y_1}.$$

we have

$$= \frac{\frac{w}{y_2}}{\frac{y_2}{Y_2} - \frac{y_1}{Y_1}}$$

The Dioptric Conditions for the Measurement of Optic Axial Angles by means of the Polarization Microscope.\*-Dr. S. Czapski lays down the following conditions in the determination of the optic axial angles of crystals: -

(1) The condenser system must have an aperture at least as large as that of the objective.

(2) The crystal plate must be parallel.

(3) The relation between the axial angles of rays in a pair of conjugate points O and O\* of the objective must be known.

(4) At O must be the crystal plate, and at  $O^*$  the eye.

(5) It is best to use an aplanatic eye-piece, and to make the path of the rays of the auxiliary Microscope "telecentrisch."

(6) For measurement with different wave-lengths the objective must be apochromatic, i. e. spherically chromatic, and corrected for the whole visible spectrum.

Geometrical Representation of the Formula for Lenses.<sup>†</sup> - M. D'Ogagne states that by a well-known construction the magnitudes occurring in the formula

$$\frac{1}{p} + \frac{1}{p^1} = \frac{1}{f}$$

may be represented by the distances in which a straight line rotating about a fixed point cuts two rectangular axes, and the distance of the centre of rotation from the centre of the co-ordinates. He remarks that this construction is more convenient if the axes are taken as intersecting at an angle of  $120^{\circ}$  instead of  $90^{\circ}$ .

Rings and Brushes .- Mr. E. M. Nelson read the following note at the April meeting :- It is a curious fact that nowhere in microscopical text-books is any account given of the method of viewing the rings and brushes which certain minerals show under polarized light.

There are perhaps some microscopists who, thinking that such objects can only be seen with a petrological Microscope, and having no desire to prosecute deep research in that direction, and being unwilling to purchase a petrological Microscope, are content to shelve the subject. Now as these beautiful objects are within the reach of all those who have an ordinary microscopical outfit, I thought it worth while to bring before the Society an explanation of a simple way of seeing them.

If you set up your instrument as if for viewing ordinary polariscope objects, not a ring or a brush will you see.

The whole point lies in the fact that it is a wide-angled telescope you require and not a Microscope at all. Once this is recognized the

- \* Zeitschr. f. Wiss. Mikr., ix. (1892) p. 130.
- + Central-Ztg. f. Optik u. Mechanik, xiii. (1892) p. 146.

683

whole thing becomes simple enough. As the Microscope has to be turned into a wide-angled polarizing telescope, all that is necessary is to screw a low power on the end of the draw-tube. As the light requires to be passed through the crystal at a considerable angle a wide-angled condenser should be employed, but it need not be achromatic. The objective I found most suitable was a 4/10 of  $\cdot 64$  N.A.;



In substage: P, polarizing prism; C, substage condenser. On nose-piece: O<sup>1</sup>, objective 4/10 · 64 N.A.; A, analysing prism. In draw-tube: O<sup>2</sup>, objective, 2- or 3-in.; H, Huyghenian eyepiece.

but a 1/4 of  $\cdot 71$  N.A., or a 1/3 of  $\cdot 65$  N.A. will do equally well. As the whole of the back lens of the objective should be visible through the analysing "Nicol" the back lens of the objective must not be too large, thus a 1/2 in. of  $\cdot 65$  N.A. would not do so well. The analysing prism may be placed either where it is in the drawing or above the eye-piece. Practically it works very well above the objective, which is the position it occupies in "ordinary microscopical outfits."

For the draw-tube a 2 in. objective and a B or C eye-piece will be found to answer admirably.

To set up.—Before screwing the objective in the end of the draw-tube, centre the light in the usual manner, the "Nicols" being turned so as to give a light field. Next fix the objective in the end of the draw-tube, open the substage condenser to full aperture, and put the mineral on the stage. Rack down the body so that the objective on the nosepiece nearly touches the crystal, then focus with the *draw-tube* exclusively. The substage condenser should be racked up close to the under side of the crystal.

#### (6) Miscellaneous.

Microscopes and Accessories at the Antwerp Microscopical Exhibition.\*—Dr. R. H. Ward, in his Presidential Address to the Microscopical Section of the Troy Scientific Association, said :— "In this department the chief exhibitors were, naturally, the manufacturers, and with only two notable exceptions they were of the 'Continental' group.

Among the whole it was evident that one, the Carl Zeiss establishment at Jena, was easily preeminent, on account of the magnitude and variety of its exhibit, the high quality of its work, and the extent and importance of its contributions to recent development of the Microscope, especially in inventing and introducing new optical glasses of high refractive indices and in the creation of the apochromatic objective. The exhibit included a large variety of the well-known Zeiss stands, with

their different classes and powers of oculars and objectives, and their outfit of numerous and ingenious accessories; also a special stand for

\* Amer. Mon. Micr. Journ., xiii. (1852) pp. 136-40.

photomicrography, a complete special photomicrographical apparatus that is small and portable, and another of great size furnished with a Schuckert electric lantern and the most elaborate appurtenances of various kinds. But the most interesting feature of their exhibit was a demonstration of the construction of a large stand and of an apochromatic objective and a compensating ocular. All the pieces entering into the construction of a No. 1 stand were displayed spread out in a case, like a picture against the wall, each piece being given in duplicate, once in the rough casting or section of tube or wire, and again in the finished form ready for assembling into the completed instrument. In another case were superb blocks of the new optical glasses of Drs. Abbe and Schott of Jena, and of the fluorite of the Oltscheren Alp. The different stag s in the construction of an 8 mm. apochromatic objective and of a compensating ocular were also displayed in proper series, all the lenses entering into the combination being shown both in the rough and the finished state. Probably every visitor would have voted with the jury in awarding the one special prize, the diploma of pre-eminence, to the Jena Company.

Next to this, three grand prizes were given, which went to London, Paris, and Germany, respectively. The English one was bid for by a solitary stand, with two or three objectives and condensers at its feet, that looked so lonesome, not to say insignificant, that to claim a leading prize for it seemed almost presumptuous. Its appearance, amidst the neighbouring cases crowded with showy apparatus, suggested at once the quiet home from which it came in Euston Road, in contrast with the showy shops where such goods are commonly displayed. But the stand made a strong competition for its not too modest claim, to be the most perfect that is made, the apochromatic objectives were judged to merit their high reputation, and the apochromatic condensers were found to give a singularly perfect illumination, and a grand prix was awarded to Powell & Lealand.

Prizes of like grade were well earned by, and awarded to, A. Nachet of Paris, and to E. Hartnack, originally also of Paris, but since the Franco-German war, now of Potsdam, Germany, both of whom exhibited a large variety of apparatus of the very highest grade, including excellent apochromatic objectives and sumptuous photomicrographic apparatus.

Of the many creditable exhibits of a somewhat more modest grade, at least in respect of prices, by far the largest was that of Wm. Watson & Sons, of London. In fact, it was one of the most interesting and commendable features of the exposition. It stood alone, except for the single P. & L. stand, as the sole representative of the English ideas and styles, while everything around it was Continental, wholly Continental. These makers, also, in developing some of their most practical stands, have made such large and good use of American ideas and experience that we are half inclined to claim a special interest in the result. We have been much interested in their efforts during recent years to develope and improve the simple and less expensive forms of Microscopes, especially in rendering the English and American type convenient and available for laboratory use, and in building up in London well-organized shops (after the method characteristic of American practice) where work of a uniformly good quality can be done by machinery at a moderate cost. Perhaps the most universally available of their more ambitious

instruments, though fortunately far from the largest or most costly, is the one lately arranged from Dr. Van Heurck's suggestions and named after him. Though of moderate size and cost, being of exactly the size that the writer would choose as a maximum, this possesses a great number of serviceable features; and, notwithstanding the unfavourable opinions that some eminent authorities have expressed on theoretical grounds, its practical working seems excellent. The jury especially complimented 'the extreme precision of all its movements:' and the writer found it unexpectedly easy with its fine-adjustment to focus a lens of N.A.  $1 \cdot 63$  upon the shell of A. pellucida, adjusting it instantly to exactly the plane of clearest vision of the dots, and leaving it there, though every one knows that, with extreme powers, it is often easy to catch glimpses, in passing, of points which can hardly be permanently focused upon and shown to other observers.

One of the strongest and most agreeable impressions made by the manufacturers' exhibit, in the aggregate, is that of the uniformity in good workmanship, and of the variety of convenient and tasty designs, by all the prominent makers.

All the manufacturers, with two exceptions, already stated, are of the Continental sort, and never before has the writer seen grouped in one room a representative collection of their Microscopes at all comparable to this. It is most interesting to note the purity in which, in so many hands, their type of stand has been preserved and the extent to which its possibilities have been developed. This style must be abundantly satisfactory, alike to the manufacturers and to their patrons, to be so freely reproduced and elaborated by so many ingenious workers, in so many diverse places, with so little deviation from the prevailing type. The interest of the display is doubled, to Americans, from the fact that from the first we have been constantly presented with the choice between adopting the English or the Continental style as the basis The friendly though often severe battle of the stands of our own. has continued throughout the memory of the present generation, and still continues to some extent notwithstanding the evident fact that the English type has entered by far the most largely into our experience thus far. Without forgetting that where the wisdom and experience of continents are concerned, the opinions of single individuals are of little importance, the writer could not do justice to the title of this paper without giving his 'impressions' on this very interesting phase of the subject. While a user of the English style for more than thirty years, and naturally with strong prepossessions in its favour, and still satisfied with it, he must admit that the neat and unpretentious Continental stands of the smaller and more simple grades become more attractive with every increase of acquaintance. It would be difficult to find anything more tempting or practical for student's laboratory work (exclusively) than the beautiful little stands exhibited by all the French and German makers, though some of the small American and English stands still seem to be equally available. On the other hand, the larger stands of the Continental type, with elaborate adjustments and numerous accessories, always produce the feeling that, notwithstanding their ingenious designs and great efficiency, they are unfortunately clumsy and somewhat overloaded, their traditional com-

636

pactness being maintained at the cost of some unnecessary inconveniences. The possibilities of the English-American stand seem to be not yet realized on the Continent; but the writer will venture the prediction that there will be a revolution in this respect some time before the next tricentennial. The reaction has evidently commenced already in respect of accessories and incidental refinements of detail. Such microscopical aids and comforts as mechanical stages, elaborate substages and substage condensers, iris diaphragms and rapid nose-pieces, which we have been using for a generation, more or less, with great satisfaction, but which we have meanwhile heard constantly denounced by partisans of the Continental method as needless luxuries and distracting toys, all these we now see introduced and given a proper prominence by the best Continental makers. 'Tis well.

As to objectives, the progress of the present day evidently centres around or stands in comparison with, the apochromatic system. Of the success of the system there is no longer a reasonable doubt, either as a scientific or as a commercial question. Characterized by the employment of new varieties of glass, of extraordinary optical properties, manufactured at Jena, and by the substitution of fluorite (natural fluor spar) for crown-glass in several of the lenses, it corrected spherical and chromatic aberration to an extent not before attained. Brought into existence by the researches and experiments of Abbe and Schott, and first successfully introduced at the Zeiss factory, in 1884, it is now adopted for their highest grades of objectives by all the above-named makers, except perhaps Watson; and new series or varieties are being constantly introduced.\* The resolution of Amphipleura pellucida in dots, which has been ably disputed and may be still doubted by some competent judges. is well within the limit of their capacity. The writer was fortunate in being afforded the rare privilege of witnessing the official trial of the objectives by the jury, at which time the Zeiss 1/10 (2.5 mm.) of N.A. 1.63, with monochromatic sunlight illumination, showed the dots (or ' beads') with beautiful distinctness and with perfect ease. They were seen at a glance over a large part of the shell at once, always alike and with remarkable freedom from any suspicion of uncertainty; and when lost by change of focus or even by carrying the instrument to another table and readjusting the light, they could be recognized instantly, and every time, by simply correcting the fine-adjustment. As the most oblique pencils able to be utilized by this objective, as employed, ought, according to the Helmholtz theory, to be able to resolve lines of about 6000 to the millimetre, and as the A. pellucida had about 3700 rows of the dots transversely and 5000 rows longitudinally, to the millimetre, this resolution, while near enough to the theoretical capacity of the lens to give an impressive demonstration of a near approach to perfection in the construction, yet does not, by exceeding its theoretical capacity, throw discredit upon either the genuineness of the resolution or the accuracy of the optical theories involved.

But the durability of the apochromatics is, most unfortunately, not equally plain. It is said that those first made were unsafe, from the

\* Near the close of the exposition, after the report on awards was adopted and closed, a new 1/12 (2 mm.) was received from Nachet and informally examined by the jury with the result of calling out from them a special note of commendation.

ease with which changes occurred in the frail phosphate and borate glasses causing the dimness known as the disease of the apochromatics. This was always freely corrected without charge by the makers, and it is believed that the glass as now made is free from this defect. The fluorite, however, must be taken with its natural defects ; and its easy cleavage renders it very fragile. In fact, it is now known that, more than thirty years ago, Charles A. Spencer, of Canastota, N.Y., recognized its tempting optical properties, and used it in the construction of objectives, one of which is still in existence; and that he abandoned the method because of the early deterioration from the cracking of the That now used in the apochromatics is not only clearer and spar. remarkably free from colour, but is evidently more durable, and, it is hoped, practically permanent. The lenses are, however, absolutely limited in output, as the world's supply of suitable spar is confined to the stock now in hand, with no known means of replenishing it. It is, therefore, none too soon to be finding some method of making high class work without it; and this is being attempted, with encouraging success, by several makers, in the construction of the semiapochromatics, made from the new optical glasses, but without the fluorite. Though not quite as free from colour as the apochromatics, their views are very distinct, and their working qualities extremely good. The resolution of *A. pellucida* in 'beads' by a semi-apochromatic is claimed to have been accomplished in Italy."

The Microscope's Contributions to the Earth's Physical History.\* —Prof. T. G. Bonney, in the Rede Lecture for 1892, takes for his text the progress in geological research which has been directly due to the revelations of the Microscope. By the side of the epoch-making discoveries of Darwin and Wallace, Bunsen and Kirchoff, he places the work in a humbler and more limited sphere of Sorby, who, in 1856, first described the results of microscopic investigations into the structure of minerals and rocks. The method employed by Sorby was, strictly speaking, not novel, for years before, in 1827, William Nicol of Edinburgh had made sections of fossil wood sufficiently thin for microscopical examination. The device, however, had not been generally applied, and to Sorby is due the credit of first pointing out its wide possibilities.

Before telling the story of microscopical research into the history of the earth's crust, the author indicates briefly the mode in which the Microscope is used in the examination of minerals and rocks. Slices are first cut by the lapidary's wheel thin enough for most of the minerals to become translucent, if not transparent. These are then examined under a Microscope of special construction, furnished with Nicol's prisms and other optical appliances.

 $U_{\Gamma}$  on the history of the two main groups of rocks the Microscope has thrown much light. For the igneous rocks it has simplified the classification and determined the mutual relations; while for the sedimentary group it has shown the true nature of their constituents and pointed out the sources from which they were derived. But it is in helping to elucidate the problem of the metamorphic rocks, of which much less was known, that the Microscope has been of the most service.

\* Nature, xlvi. (1892) pp. 180-4.

The author deals at length with this portion of his subject, and shows how the Microscope has assisted in the attempt to determine the history and mutual relation of these rocks. One of the most important results within the last few years has been the demonstration that without exception these crystalline schists are very old, all probably older than the first rocks in which traces of life have been found. The conclusion at present arrived at is that "the environment necessary for changing an ordinary sediment into a crystalline schist existed generally only in the earliest ages, and but very rarely and locally, if ever, since Palæozoic time began."

The crystalline schists then are the relics still preserved to us of the early days of this earth's history when the temperature near the surface was still very high. Since that time the zone for marked mineralogical changes has been continually sinking until at the present day it has reached a depth practically unattainable. "The subterranean laboratory still exists, but the way to it was virtually closed at a comparatively early period in the earth's history."

In conclusion the author considers that the progress made since the Microscope was pressed into the service of geology augurs well for the future, and inspires the hope that we shall at last learn something of the history of the earliest ages "when the earth had but lately ceased to glow, and when the mystery of life began."

## B. Technique.\*

Microtechnique of Vegetable Objects.<sup>†</sup>--Dr. L. Klein describes at length some points in the technique of vegetable histology, and considers them under the heads of imbedding in celloidin and paraffin, the media suitable for mounting, and the most convenient staining solutions.

For celloidin imbedding, the object having been thoroughly dehydrated by immersion in absolute alcohol in a Schulze's dialyser, is soaked for 6–10 hours in a mixture of equal parts of ether and absolute alcohol. The piece, the sides of which should not exceed 3–5 mm. long, is then placed in a thin solution of celloidin (about 5 per cent.) in equal parts of ether and absolute alcohol. In this solution, contained in tightly stoppered bottles, the object remains for at least three days. After this time the celloidin solution is thickened by slow evaporation of its solvents, and this is effected first by substituting a cork for the glass stopper, and afterwards by inserting strips of paper between the neck of the bottle and the stopper. It is finally inspissated by direct evaporation, and then by immersion in 60 per cent. spirit. The celloidin block, the section surface of which should not exceed a square centimetre, is fixed on cork or wood by means of a thin layer of celloidin solution of the consistence of syrup.

The surface of both the cork and section block should be previously moistened with ether, and the two parts having been tightly squeezed together, the mass will be sufficiently adherent in 10–15 minutes for

\* This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes; (4) Staining and Injecting; (5) Mounting, including slides, preservative fluids, &c.; (6) Miscellaneous.

† Jahrbücher f. Wiss. Botanik (Pringsheim), xxiv. (1892) pp. 1-57. 1892.

3 а

cutting. Instead of squeezing the two—cork and block—together, they may be fused firmly by immersing them in 60 per cent. spirit for about a day. All the foregoing steps are to be carried out in glass vessels, but the formation of the section block by pouring a thickish solution of celloidin into paper cases, and then, having properly oriented the object, hardening the mass by immersion in 60 per cent. spirit, is also advised. In any case the consistence of the celloidin block should be about equal to that of cartilage.

In sectioning an object imbedded in celloidin, it is necessary to use an immersion microtome, or, at any rate, to employ some means for keeping the knife moist. This may be effected by a dropping apparatus filled with 60 per cent. spirit. It is not at all necessary to remove the celloidin from the sections, and these, after they have been mopped up with blotting-paper, are easily made to adhere to the slide by exposing them to ether vapour.

The further treatment of the sections depends on whether they are to be mounted in an aqueous or resinous medium. In the former case the dilute spirit is removed by immersion in water; in the latter by treating them with 90 per cent, spirit. The author's remarks on paraffin mostly refer to the treatment of sections which have been imbedded in paraffin. He advises that all manipulations should be carried out on the slide on which the sections are to be deposited on removal from the knife. The technique of these steps is, of course, the same as that usually adopted; the paraffin having been dissolved out, the ultimate treatment of the section will depend on the character of the medium in which the section The mounting medium may be aqueous or resinous, is to be mounted. and if the former, then it may remain fluid (acetate of potash, chloride of calcium solution), or may set (glycerin jelly), while resinous mounts require the preparation to be dehydrated. Hence the effect on the section will vary much, and, therefore, which course of the three should be adopted must be decided by the nature of the tissue in the section.

Different kinds of mounting media are then passed in review and the first mentioned are chloride of calcium and acetate of potash, both of which are pronounced to be suitable for delicate parenchyma and for tissues rich in protoplasm, but the use of these fluids is to be avoided, owing to the extra trouble such preparations involve. Glycerin jelly receives commendation, as it may be used for stained objects, does not require to be perfectly cleared, nor to be ringed round. Media, the principal constituent of which is gum, are then discussed : of those noticed may be mentioned Farrant's medium (of which the author admits his practical ignorance) and Hoyer's media, one of which, made of gum, chloral hydrate, and glycerin, is intended for preparations stained with logwood and carmine, while the other is composed of gum and acetate of potash solution. Both these fluids are of the consistence of thick syrup, and as they dry at the edge, ringing round the cover-glass is not necessary.

Resinous mounting media are colophonium, dammar, and balsam, the first two of which are best dissolved in turpentine, as too volatile solvents are inadmissible on account of the crystalline structure of these resins. Colophonium does not appear to possess many advantages, although one may be mentioned: it is little sensitive to a residuum of water, and therefore complete dehydration is not absolutely necessary. With regard to dammar and balsam, it is only necessary to say that the latter is probably the more useful, owing to its high refractive index, although there seems little to choose between the two media. For this technique balsam is dissolved in chloroform xylol or turpentine, dammar in benzol, or xylol.

For staining vegetable tissue, Bismarck brown holds a prominent place: a small quantity of this pigment is shaken up with spirit, and half the bulk of water added. Only a clear filtrate must be used. This solution is allowed to act for  $\frac{1}{2}$ -3 minutes, and appears to give great satisfaction. The next pigment is safranin: the solid pigment is dissolved in spirit, and an equal volume of water added.

Alum and borax carmine are then discussed, both of them are made up according to Grenacher's formula: both have their merits, but the latter has to be differentiated with an acid solution.

Delafield's alum hæmatoxylin also is extremely serviceable and easy of manipulation, and if over-staining occur, the excess is easily removable by means of a faintly acid solution. Notwithstanding the contrasts developed by the use of different pigments or the appearance of different shades, indicating the differences of chemical reaction or of structure, the author advises that these should be compared with unstained sections mounted in the gum and acetate of potash solution.

#### (1) Collecting Objects, including Culture Processes.

Apparatus for Cultivating Anaerobic Micro-organisms on Solid Transparent Media.\*-..Dr. A. Trambusti describes an apparatus which he has devised for cultivating and examining anaerobic microbes.

It is made of glass and consists of two parts, the lower of which resembles an inverted funnel while the upper one is cylindrical. In the latter are two openings, the top one tightly closed with a stopper while that at the bottom is very small. Inside the cylinder is a smaller one which is in communication with the funnel-shaped flask or lower portion of the apparatus. The apparatus is used as follows:-The medium, already inoculated, is spread on the bottom of the flask and then this is closed by placing the cylinder on it. Into the latter is then poured as much of the ordinary pyrogallate of potash solution as may be necessary to absorb all the air in the apparatus. The stopper having been put in, the whole apparatus is placed in the thermostat. Two grm. of pyrogallic acid to 15 ccm. of 1 in 10 potash solution are sufficient to extract all the oxygen.

Colonies of anaerobic micro-organisms are said to thrive very well in this apparatus and are quite easily isolated. The apparatus has the further advantage that the growth of the colonies is easily examined through the thin bottom and their shape studied just as in Petri's flasks.

If the pyrogallate solution be shaken up occasionally the absorption of the oxygen is accelerated.

Apparatus for Cultivating Anaerobic Bacteria.<sup>†</sup>—Prof. M. Ogata describes a simple and easily made apparatus which he has used successfully for the cultivation of anaerobic microbes for some years.

3 A 2

<sup>\*</sup> Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 623-4 (1 fig.).

<sup>†</sup> Tom. cit., pp. 621-3 (2 figs.).

## 692 SUMMARY OF CURRENT RESEARCHES RELATING TO

A test-tube filled in the usual manner with gelatin or agar and plugged with cotton-wool, is drawn out by means of the blow-pipe flame just below the cotton-wool plug, until it becomes longer and narrower than the tube of a Liborius' apparatus. This heating does not affect the Then a small glass tube is drawn out into a capillary tube, a gelatin. few centimetres of the original being left, so that the capillary portion is longer than the test-tube. The latter, after the cotton-wool plug has been replaced, is sterilized by heat. The nutrient medium having been liquefied by immersing the tube in lukewarm water, is inoculated by means of the capillary tube. The wide part of the capillary tube is then connected with the apparatus for developing gas (H, CO<sup>2</sup>, &c.) and its end pushed close down to the bottom of the test-tube. As the gas enters the air is driven out, and when this is effectually completed the capillary tube is withdrawn and the test-tube sealed up at the narrow part.



Apparatus for Evaporating Fluids at Low Temperatures.\*-Dr. S. v. Dzierzgowski and Dr. L. v. Rekowski have devised an apparatus \* Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 685-9 (3 figs.).

for evaporating down nutrient media so as to avoid the danger of exposing the fluid to contamination during the process. The apparatus will evaporate a watery fluid at a temperature of 23° to dryness in quite a short time, and keep it germ-free; it consists of two parts: a conical glass vessel holding 3-4 litres; in the base, which is uppermost, are two openings, and the apex of the cone is also open; all three openings can be closed with caoutchouc plugs. This vessel is placed inside another made of brass and of similar shape. The latter is supported on an iron stand, and is fitted with glass windows for observing the evaporating fluid, a thermometer, and a thermo-regulator. Besides the foregoing are required caoutchouc plugs, bent glass tubes, a Chamberland's filter, Wolff's bottles, with a manometer, a Liebig's cooler, and a water suction pump.

The arrangement of the apparatus is shown in fig. 82, and after all the various parts have been carefully sterilized, the glass vessel A, with its three openings closed, is placed inside the metal case B. Above the opening 3 is a firm rubber valve. The plugs are then removed from 2 and 3, and these apertures connected with D the filter and G the cooler. The other end of G the cooler is in its turn connected with the water pump J through the mediation of the Wolff's bottles  $H^1H^2$ .

The water-tap is now turned, and the pump beginning to act, exhausts the air from the apparatus. The fluid to be filtered is then poured into the filter, and in about seven minutes the first drops begin to fall into A. As soon as the desired quantity is obtained, the process is stopped by the clamp L. The pan B is then filled with water and the burner N underneath lighted. The temperature of the water-bath is to be regulated for 38°. In twenty-four hours about two litres of fluid will have been filtered.

This part over, air is allowed to enter by removing first the Chamberland's filter and replacing it by a glass tube constricted at one place and stuffed with cotton-wool. The screw L is then loosed and air, filtered through the cotton-wool, enters the apparatus.

New Method for Ascertaining the Temperature of Sterilizing Ovens.\*—On account of the difficulty of regulating the thermometer M. Quénu uses a small tube filled with an easily fusible mass for indicating the temperature of his sterilizer. Sulphur which melts between 112° and 117°, benzoic acid, and an alloy of bismuth and tin which melts between 130° and 143° were found to answer best. The author states that the method is convenient but costly.

Cold-sterilized Albuminous Nutrient Media. $\dagger$ —Dr. A. Reinsch makes milky nutrient media in the following manner. 500 ccm. of fresh cow's milk mixed with 1.0 grm. of NaHO are well shaken up in a separator-funnel and then allowed to stand for 48 hours at a temperature of about 18°. At the end of this time the fatty matters are collected in a creamy layer on the surface of the fluid, the now pretty clear fluid is transferred to another filter and then shaken up with 250 ccm. of ether. At the end of 48 hours the ether has separated from the clear but slightly opalescent fluid. The latter still contains a considerable

\* La Semaine Méd., 1892, p. 203. See Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) p. 40. † Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) pp. 30-2.

quantity of ether, which is removed by heating the fluid, in flasks plugged with cotton wool, up to  $50^{\circ}$  and then placing it under an air pump whereby the ether is evaporated in 3-4 hours. This sterile and fat-free milk may be used as a cultivation fluid or rendered solid as follows:—Two parts of this unfatted milk are mixed with one part of a 3-4 per cent. agar solution at a temperature of  $50^{\circ}$  and then placed in test-tubes. The tubes should then be placed in an incubator for a few days in case any air germs may have infected them. Made in this way milk-agar is quite transparent pale yellow, and with reflected light faintly opalescent.

Dr.  $\hat{R}$ . Wollny\* has devised a method whereby many of the inconveniences unavoidable in the customary methods of sterilizing cultivation media are obviated. He has found ordinary ethyl-ether to be an excellent agent for this purpose and the subsequent removal of which does not present the slightest difficulty. Not only does it not affect the chemical composition of the fluids on which it exerts a sterilizing influence, but it also aids in removing from them fatty matters usually so detrimental to cultivation media.

The juice of finely chopped-up meat, fish, liver, potatoes, &c. (or blood, urine, milk), which has been expressed or extracted with water, is mixed with 10 per cent. of ether and placed in a closed vessel. If any acetic acid be present from the oxidation of the ether it must be neutralized with an alkali. The fluids are then cleared by decantation or filtration or may be thickened by the addition of 3 per cent. agar solution or 15-20 per cent. gelatin solution. The ether is then removed by placing the nutrient fluid in a flask closed with cotton-wool; the flask is next heated to  $35^\circ$ - $40^\circ$  and placed under the receiver of an air-pump. The fluid is then ready for use, although at this stage it may be mixed with agar-gelatin or soda solution. None of the albumen is lost and it is perfectly unchanged.

Many nutrient solutions prepared in this way, such as meat, liver, potato, are of a rather dark colour, but they are usually quite transparent enough for cultivation purposes; others, such as extracts of intestine, fish, milk, are perfectly clear and transparent.

In preparing milk by this method it is necessary to use a larger quantity of ether in order to completely remove all the fat, and also to add caustic soda solution if a perfect and transparent solution is desired.

Growth of Bacteria on Acid Nutritive Media.<sup>†</sup>—The most prevalent notion as to the reaction of cultivation media is, says Herr G. Schlüter, that an alkaline or neutral reaction is almost absolutely necessary. In order to show that an acid reaction is not so very inimical to bacterial growth, the author made a series of experiments with some of the best known bacteria such as *Bac. typhosus*, *Bac. anthracis*, *Staph. pyogenes aureus*, Friedlaender's pneumonia-coccus, the coccus of erysipelas, and others. The basis of the medium was ordinary gelatin or isinglass mixed with 1.25 grm. pepton, 1.25 grm. NaCl, and 250 grm. water. To this mixture were added acids (lactic, tartaric, citric, acetic, hydro-

† Tom. cit., pp. 589-98.

<sup>\*</sup> Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 752-6.

chloric) or alum in various degrees of concentration. The cultivations were made in test-tubes at temperatures varying from  $16^{\circ}-23^{\circ}$ .

The results, carefully tabulated and recorded, amount to this, that a large number of bacteria grow on acid media, some indeed very well, provided that a certain degree of acidity be not exceeded. The maximum acidity of the medium was found to vary extremely for each different species. The only Schizomycete which would not grow at all on any acidified medium was the micrococcus of erysipelas, while the *Bacillus anthracis* grew even when the medium contained 0.2 per cent. of lactic acid, and with 0.2 per cent. of alum its growth was better and more rapid than on neutral media. Nor was the virulence of the anthrax diminished, as was proved by inoculation experiments on mice.

It would appear that the biological characteristics of some fission fungi are brought out very clearly on acid media; among these may be mentioned the bacillus of typhoid and the bacillus of blue milk; the latter grown on isinglass decoction, to which 0.2 per cent. of lactic acid had been added, developed its well-known pale blue hue, a phenomenon which does not occur on alkaline substrata.

Pure Cultivation Methods and Specially Koch's Plate Cultivation and the Limit of Error of this Method.\*—In the first part of his article Herr J. C. Holm passes in review the history of pure cultivation methods and points out that pure cultivations may serve two different ends, viz. for examining the morphology of a micro-organism and for making physiological experiments with it.

The second part is an experimental study on the limits of error in Koch's plate cultivation method, though in reality it only deals with the problem of how many pure cultivations arising from a single cell can be obtained by isolating yeast cells on gelatin plates, bacteria being left out of consideration altogether.

The third part deals with the numerical variability of yeast cells capable of developing in wort gelatin, according as these cells are taken at the beginning or end of the fermentation process, and also discusses the gelatin media suitable for yeast cells. It is here stated that 4.5 per cent. of the yeast at the beginning and 25.5 per cent. of that at the end of the fermentation process is incapable of development, and that gelatin made up with beer wort gives the best results.

**Preparing Catgut.**<sup>†</sup>—Herr Braatz has made some experiments for the purpose of showing how fat prevents disinfection. Pieces of catgut  $1-1\frac{1}{2}$  cm. long were sterilized in dry heat of 140° for 3 hours; these were then infected with fresh anthrax spores and preserved dry. He then succeeded in showing that oil had a detrimental effect in disinfecting with sublimate solution.

Hence if catgut is to be effectually disinfected it must be deprived of fat, and this is best done by means of ether, and after this, treatment with sublimate is the best procedure.

The author further made some experiments as to the sterilization

\* CR. des Travaux du Laboratoire de Carlsberg, iii. (1891) pp. 1-23. See Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 576-7.

† Brun's Beiträge zur Chirurgie, vii. (1891) No. 1. See Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 627-8.
of catgut by means of heat and used for this purpose a Liebig's bath filled with olive oil. Catgut left at 140° for 3–4 hours was found by cultivation and by experiment to be perfectly sterile.

The author recommends two methods for preparing sterilized catgut. (1) Raw catgut rolled round a glass cylinder is unfatted by immersion in ether (ethyl) for 1-2 days. The complete absence of fat is easily ascertained by pouring out a little ether in a watch-glass and allowing it to evaporate; if there be no residue then the fat is completely removed. The ether should be renewed once or twice. From the ether the catgut is removed straight away to sublimate solution 1-1000 wherein it remains for 24 hours, after this it is preserved in absolute alcohol.

(2) Raw catgut having been unfatted as in the foregoing method is rolled in bibulous paper and heated in an oil bath for 4 hours. It is then placed in absolute alcohol.

### (2) Preparing Objects.

Simple Method of Substituting Strong Alcohol for a Watery Solution in the Preparation of Specimens.\* — Prof. W. A. Haswell suggests the following method :—" Lo Bianco has in the last part of the 'Mittheilungen aus der Zoologischen Station zu Neapel,' published an account of the methods which he follows in preparing those marvellous specimens of marine invertebrates for which the station has long been famous all over the world. Many of the methods described have now been known to zoologists for some time, i. e. many of the methods of killing and fixing; it is more perhaps, on account of the information which it gives us, as the result of a long series of trials, as to what reagents are best adapted to each special group, with the best modes of application in each case, than as giving any entirely new formulæ, that the paper is of value.

As is well known, marine animals of different groups require to be dealt with in very different ways in order that we may preserve them in anything approaching to their natural form. Some may be taken by surprise, if we may use the expression, and killed so suddenly by some powerful poison that they remain fixed in a life-like shape. Others must be narcotized or paralysed by some such reagent as chloroform, weak alcohol, or chloral hydrate, before the killing and fixing agent is used.

Whatever be the method of killing and fixing employed, there is in all delicate organisms a difficulty experienced in preventing shrinkage during the later processes which the specimens have to undergo before reaching the strong alcohol stage. In the most admirably fixed specimens shrivelling will often appear when alcohol is applied. This difficulty is partly overcome, with great pains, by using a series of alcohols of ascending degrees of strength. But the result of this mode of procedure is not by any means always satisfactory.

Dr. Cobb, in a paper read before this Society,<sup>†</sup> has described a method by which, in the case of small organisms, the shrinkage due to change from one fluid to another of a different density may be reduced to a minimum. In his differentiator we have an instrument of

<sup>\*</sup> Proc. Linn. Soc. N. S. Wales, 2nd series, vi. (1891) pp. 433-6.

<sup>†</sup> Op. cit., v. p. 157. See this Journal, 1890, p. 821.

admirable simplicity for insuring this result. But I have found that in practice the use of the differentiator involves a considerable expenditure of time. To get a specimen from distilled water to 90 per cent. alcohol for example, no fewer than eleven different mixtures of water and alcohol have to be made up and poured into the reservoir tube.

A simple piece of apparatus which I have devised does away entirely with this—the gradual substitution for one another of the two fluids of different densities being effected automatically. An obvious mode of meeting the difficulty suggests itself at once. Why not have the second fluid falling into the first drop by drop, mixing thus very gradually with it and eventually replacing it? The difficulty in the way of this is that as each drop of the much lighter liquid enters the denser, violent though circumscribed currents are produced which are damaging to the delicate organisms we are dealing with.

The requisites for the method about to be described are—several reservoirs of glass or earthenware fitted with glass taps and having

each a capacity of a gallon or more, some wide-mouthed bottles of a variety of sizes, fitted with perforated india-rubber stoppers, and some lengths of glass and india-rubber tubing.

Two bottles of similar size are connected together by tubing in the way represented in the woodcut. One of these A we call the mixing bottle; the other B contains the objects, and must have a capacity (fig. 83) equal to at least a hundred times the bulk of the latter. The objects are in fluid 1, and it is desired to substitute fluid 2. Both bottles are filled, or partially filled, according to circumstances, with fluid 1, and bottle A is connected with a reservoir of fluid 2. It is somewhat difficult by means of a tap to regulate the flow so that, let us say, one drop in five seconds will pass out of the reservoir; and it is much more convenient to effect this by intercalating in the supply pipe a section of glass tubing drawn out to the required degree of fineness (represented in the figure as disconnected from the proximal portion of the supply tube). The rate of flow through this narrow section of

Fra. 83.

the tube can be further regulated by raising or lowering the reservoir or the mixing bottle, thus altering the pressure. With bottle B is connected an overflow tube. Above the narrow section of glass tubing in the supply pipe it is well to have a piece of filter paper stretched across the mouth of the piece of tubing in the form of a diaphragm, and held in place by the overlapping india-rubber tubing. This prevents the possibility of the narrow part of the tube being choked up by any minute particles.

Fluid 2 thus enters into the mixing bottle at an extremely slow rate of flow, and becomes completely diffused, at first in extremely minute quantity, through fluid 1. The fluid from the mixing bottle is meanwhile entering bottle B at the same extremely slow rate, and it is obvious that with two fluids that readily mix, fluid 1 may be made to replace fluid 2 in bottle B with the required excessive slowness and regularity.

In the case of some of the liquids used in fixing and preserving, it is not necessary to use such a precaution as this. We may substitute saturated solution of corrosive sublimate for sea-water without the least risk of damage to the most delicate structures—the specific gravity of the two being very nearly the same.

Similarly distilled water may be at once substituted for osmic acid solution, or 1 per cent. chromic acid, or other fluid that does not differ at all widely from water in specific gravity. But with certain fluids the gradual substitution is necessary, and it is above all necessary in replacing water or a watery solution by alcohol, and this, in the case of large specimens intended for museum purposes as well as smaller objects, can very conveniently be carried out by the simple apparatus I have described above.

Another method of effecting this substitution is the one devised by Schultze; and this seems to possess some decided advantages, at least for small objects. Schultze places the objects which he wishes to transfer from water to alcohol in a tube full of water, plugged at one end, and closed at the other by a diaphragm of chamois skin. The tube is placed in a vessel of alcohol and left there until by a process of diffusion through the diaphragm the water in the tube becomes completely replaced by alcohol, the same material being used for the diaphragm. The time which will be occupied before complete substitution takes place will vary with the capacity of the tube and the diameter of its orifice; and a series of experiments and calculations would have to be made before this method could be used with the assurance of good results. Should it be desired to have the specimens in absolute alcohol at the end of the process, some calcined sulphate of copper may be placed in the outer vessel."

Investigation of Blood of Amphibia.\*—Dr. A. B. Macallum experienced great difficulty in finding a reagent which would show the presence not only of hæmoglobin, but of its antecedent, if such existed. Of the dyes belonging to the aromatic group of organic compounds eosin was the only one which was found to be useful. A reagent of great service was the staining fluid of Shakespeare and Noring which the author calls the Indigo-carmine Mixture. As he makes it, it consists of a mixture of equal volumes of two solutions; one consists of carmine 2 grm., borax 8 grm., and distilled water 100 c.c. The other is com-

\* Trans. Canadian Institute, ii. (1892) pp. 222-8.

posed of indigo-carmine 8 grm., borax 8 grm., and distilled water 100 ccm. The section to be stained is left in the fluid for 15 minutes, then plunged in a saturated solution of oxalic acid for 10 minutes, washed in distilled water, dehydrated with absolute alcohol, cleared in pure xylol, and mounted in benzole balsam. Essential oils should be avoided as they appear to oxidise the indigo-carmine, and cause the stain to fade.

For the hardening of the tissues preparatory to cutting, small portions should be half an hour in a saturated solution of carmine sublimate, or 5 days in Erlicki's fluid, or 24 hours in a 1/5 to 1/3 per cent. solution of chromic acid, 5 hours in a saturated solution of pieric acid, or 2 to 5 hours in 1 per cent. solution of osmic acid. They must then be washed in distilled water, and put in 50 per cent. alcohol for 2 hours, in 70 per cent. for 24 hours, and finally in 95 per cent. The pieces may be imbedded in mucilage and sectioned in the freezing microtome, or by the chloroform method in paraffin. The great value of these preparations lies in the fact that hemoglobin is stained grass-green or greenish-blue, while other proteid elements are coloured red, save a few about which there can be no mistake.

Alum-hæmatoxylin, in which ammonia-alum is dissolved to saturation, and Czokor's alum-cochineal were also of great value in the study of the hæmatoblasts of the larvæ of *Amblystoma*.

Cover-glass preparations of blood were fixed either in the fumes of osmic acid (1 per cent. for 2 hours), or by a saturated solution of corrosive sublimate or picric acid, or by Erlicki's fluid. The fixation was completed as usual with alcohol, and the various dyes already mentioned were used for staining the preparations.

Examination of Teleostean Ova.\*-In his study of the mesoderm of Teleostean fishes Mr. E. R. Boyer made use of the oviparous Cyprinodont Fundulus heteroclitus. The ova were killed at intervals increasing from one hour in the younger to eight hours in the older stages. The killing reagents used were Perenyi's fluid, Kleinenberg's picro-sulphuric mixture, and a solution of 0.25 per cent. osmic acid, followed by Whitman's modification of Merkel's fluid. The ova were next carried through grades of alcohol to 90 per cent. Those preserved with Perenyi's fluid proved to be most satisfactory in the earlier, and those with Kleinenberg's mixture in the later stages. The best staining results were obtained by the use of Kleinenberg's hæmatoxylin in toto for 20 to 24 hours, and decolorizing with 70 per cent. acidulated alcohol for about 2 to 4 hours. With osmic material, however, the best results were attained by the use of Czokor's cochineal for 10 to 12 hours. The embryos were removed from the yolk under the dissecting Microscope, dehydrated, penetrated with clove or cedar oil, followed with paraffin at a temperature of 55° C., imbedded in paraffin in the usual way and cut into sections by a Thoma or a Cambridge rocking microtome.

Study of Germinal Layers of Petromyzon.<sup>†</sup>-Mr. S. Hatta hardened the eggs and larvæ of *Petromyzon* partly in Kleinenberg's picro-sul-

<sup>\*</sup> Bull. Mus. Comp. Zool., xxiii. (1892) pp. 93 and 4 (8 pls.).

<sup>+</sup> Journal Coll. Sci. Imp. Univ. Japan, v. (1892) p. 130.

phuric acid, and partly in corrosive sublimate. A few larvæ were also killed in Flemming's solution. The sublimate specimens gave the best results. Picrocarmine was found to be the best staining reagent, as, being a nuclear stain and not affecting the yolk-granules, it made observation comparatively easy.

Preparing Liver of Gastropoda and the Reconstruction of Organs.<sup>\*</sup> —For his observations on the morphology of the liver of Gastropoda, Dr. H. Fischer made use of sections of embryos; these were imbedded in paraffin and previously fixed with saturated aqueous solution of sublimate, with alcohol or with picrosulphuric acid. This method, though not altogether irreproachable from a histological standpoint, is quite satisfactory for morphological purposes, as it not only allows of thin sections being made, but of the object being reconstructed from them. In reconstructing an object it will be found very useful to draw with the camera the outlines of the embryo after it has been fixed, as the possession of this contour renders it easier to reconstruct the object from drawings of its sections.

The usual method of reconstructing a model of the object in relief is to make copies of the different sections in wax. Each wax plate should have a thickness equal to the product of the thickness of the section of the enlargement of the drawing. The outline of the object of each section is then drawn on these plates with the camera, and then the superfluous parts cut away. The plates having been joined together, an enlarged model of the original object is obtained.

It is very important that the sections should be kept in series, or that some method for finding out the proper position of the individual plates should be adopted. If there be no possibility of doing this from the shape of the object or of its component parts, then marks must be placed on the surface of the paraffin block. These marks may be made by scratching a shallow furrow on one or more faces of the block, or by including a hair. The former is better, as some colour, e. g. black lead, may be worked in.

When the organs are very thin or of very irregular shape, wax models are too fragile, but the difficulty may be avoided by cutting out the organs to be represented from the plate, so that the organ is represented by a cavity, and this may be reproduced, after the model is finished, by means of a plaster cast.

Investigation of Nephridia of Prosobranchs.<sup>†</sup>—Dr. R. v. Erlanger dropped living specimens of *Patella* and *Fissurella* into Kleinenberg's picro-sulphuric fluid with a few drops of osmic acid, or into a mixture of sublimate 5 per cent. in sea-water 3 parts and 1 part of glacial acetic acid. Flemming's chromosmic acetic fluid was found to make the tissues too brittle. In *Trochus* it was necessary to use a different method, on account of the stout shell and operculum. The specimens were, therefore, put into sea-water and 1 per cent. absolute alcohol in order to draw the anterior half of the body out of the shell. After a day or two the animals are paralysed without being killed. The fixing

\* Bulletin Scientifique de la France et de la Belgique (Extr. from), xxiv. (1892) 87 pp. (7 pls.).

† Quart. Journ. Micr. Sci., xxxiii. (1892) pp. 589-91.

liquid will now easily penetrate the shell and the mantle cavity. The chief stain used was alum-carmine, used to stain in bulk. It is convenient to remove the foot and other organs with a razor or a pair of scissors, as a great deal of time and trouble may be saved, and the stains penetrate more easily. The sections were cut with Yung's microtome, after imbedding in chloroform and paraffin. At Prof. Lankester's suggestion use was also made of the injection method, though the author is strongly prejudiced against it, as he thinks it very likely to mislead. He used soluble Berlin blue, and injected by blowing the injection through a fine glass pipette with the mouth; these injections confirmed the results obtained by dissections and sections. But the use of syringe and strong pressure resulted in the breaking of the walls at various points.

**Preparation of Nudibranchs.**<sup>\*</sup>—Most of the Nudibranchs whose cerata have been studied by Prof. W. A. Herdman and Mr. J. A. Clubb were killed and fixed with Kleinenberg's picric acid, stained with picrocarmine, passed through graduated alcohols, imbedded in paraffin, and cut with the Cambridge rocking microtome. The specimens of *Hermæa dendritica* were obtained at Plymouth by Mr. Garstang, who plunged them for a moment, while alive, into glacial acetic acid, transferred them to a saturated solution of corrosive sublimate for half an hour or so, and then passed them through grades of alcohol.

Study of Development of Limulus longispina.<sup>†</sup>—Mr. Kamakichi Kishinouye fixed the eggs of L. longispina by heating in water to 60° or 70° C., or by plunging into water of that temperature. After cooling they were transferred to 70 per cent. alcohol, where they were left for one or two days. Eggs in early stages were in some cases pierced through into the yolk with the point of a fine needle at two or three points, care being taken not to hurt the germinal disc. These perforated eggs were left in 70 per cent. alcohol for one or two days more, and were afterwards dehydrated in increasing grades of alcohol. For the surface view of the embryo the ventral plate was peeled off the underlying yolk of the preserved egg, and was stained by borax-carmine, washed in acidulated alcohol, and imbedded in Canada balsam, after dehydration and clarification.

Eggs which it was proposed to cut into sections were stained with borax-carmine or hæmatoxylin *in toto*; owing to the abundance of the yolk the process of section-cutting was very troublesome. Very good sections were obtained by the use of the celloidin-paraffin method.

Investigation of Nectonema.<sup>‡</sup>—Mr. H. B. Ward found that the resistant cuticle of this worm, which hinders the passage of most fluids, and its strong tendency to curl in the killing fluid were great obstacles in the successful preservation or sectionizing of specimens. The best reagents were found to be a saturated aqueous solution of corrosive sublimate and Perenyi's fluid heated to about 60°. Picro-nitric acid gave nearly as good results. The curling of the specimens may be largely prevented by straightening the worm gently with the fingers,

t Bull. Mus. Comp. Zool., xxiii. (1892) pp. 136 and 7.

<sup>\*</sup> Quart. Journ. Micr. Sci., xxxiii. (1892) p. 544.

<sup>+</sup> Journal Coll. Sci. Imp. Univ. Japan, v. (1892) pp. 56 and 7.

and dropping it suddenly into the warm killing reagent. Eight carmine solutions were tried, but the only one which was useful for staining was Mayer's hydrochloric acid carmine, after prolonged immersion. All hæmatoxylin solutions stain well, but require more time than usual.

In imbedding in paraffin it is necessary to keep the temperature low. Series cut in paraffin of 50°-52° were in all respects most successful. The infiltration must be complete, but a long immersion in paraffin renders the objects very brittle. Maceration was tried on preserved material with little success. Great assistance was derived from the study of portions of the body which had been cleared in clove-oil before staining.

Examination of Lucernariidæ.\*-Dr. G. Antipa found picrocarmine and Beale's carmine the best staining reagents, but good results were obtained with osmic acid and gold chloride; for the last Löwit's or Viallanes' methods may be used. For the study of the separate epithelial cells macerations were chiefly effected by Hertwig's mixture of 0.05 per cent. osmic acid 1 part, and 0.2 per cent. acetic acid 2 parts.

Method of Examining Blood, Bone-Marrow, and Body Juices.<sup>+</sup>---Dr. R. Muir makes blood films on cover-glasses, and then before the film can dry it is placed face downwards on the surface of a saturated solution of sublimate with 3/4 per cent. of sodium chloride added, for about half an hour. If the solution be heated to 50° C. it acts better. The cover-glass is next washed in 3/4 per cent. salt solution, and then passed through successive strengths of alcohol. After this it may be stained in the usual manner and with the staining solutions used for sections.

Bone-marrow is lightly dabbed on the cover-glass, not spread, so as to form a thin layer, and then treated as above.

New Method of Preparing Dentine.<sup>‡</sup> — Dr. W. Lepkowski finds that the following modification of Ranvier's fluid serves excellently well for simultaneously softening and staining dentine and also bone.

In a mixture composed of 6 parts of a 1 per cent. watery solution of gold chloride and 3 parts pure formic acid are placed pieces of teeth 1/2 - 3/4 mm. thick.

The little bits of teeth, obtained by means of a hand-saw, have, after an immersion in the foregoing fluid for 24 hours, the consistence of cork, and can be easily cut with a razor. When removed from the solution the pieces of teeth are first washed with distilled water, and then placed in a mixture of gum arabic and glycerin for 24 hours. On removal from this last reagent, the pieces are again washed with distilled water and then with alcohol, after which they may be imbedded in celloidin or paraffin and sectioned.

This procedure is much better adapted for fresh teeth than for those which have been kept for any length of time.

The method is also applicable to bone, thin slices of which are decalcified within 24 hours.

Preparation of Vegetable Tissues.§-Mr. A. Flatters gives detailed instructions in the use of the microtome and the cutting of sections

\* Zool, JB., vi. (1892) p. 379. † Journ. Anat. and Physiol., xxvi. (1892) p. 393.
‡ Anat. Anzeig., vii. (1892) pp. 274-82 (1 pl.).
§ Trans. Manchester Micr. Soc., 1891, pp. 38-47 (1 pl.).

of vegetable tissues, the preparation of leaf- and flower-buds, the mounting of celloidinized sections, and the staining of vegetable tissues with logwood, and with borax-carmine and iodine-green. Formulæ are given for preparing Kleinenberg's hæmatoxylin, picrocarmine, boraxcarmine, and glycerin-jelly.

## (3) Cutting, including Imbedding and Microtomes.

Strasser's Ribbon Microtome.\*—Prof. H. Strasser describes a larger model of his so-called "Schnitt-Aufklebe" Microtome.<sup>†</sup>



The details of the instrument are seen in fig. 84. The knifeholder MS runs on a double slide-way, and the object-carrier T is raised

\* Zeitschr. f. Wiss. Mikr., ix. (1892) pp. 1-13. + See this Journal, 1891, p. 281.

by the micrometer screw M. There is a catch arrangement at E for determining the thickness of the sections.

The arrangement of the paper band, to the under surface of which the sections are made to adhere, is similar to that previously described. From the roller I the band passes through the guiding loop h K, thence through the apparatus for smearing the paper, and on to the knife-edge where it is directed upwards to the clamp v K, to which are attached two strings passing over the pulleys r and kept taut by a weight.

The apparatus for smearing the paper consists of a box containing three rollers. Roller III. in front, which is of metal and of small diameter, serves to keep the paper band in the plane of the knife-edge. The two hinder rollers are of wood covered with felt. Roller II. is set in motion by the advancing paper band and transmits the motion to roller I.; the latter takes the adhesive material and conveys part of it to roller II. which spreads it uniformly on the under surface of the paper band.

The roller-holder WS and knife-holder MS are similar in construction. In both two side pieces engage underneath in the horizontal slide-way, and are connected above by a strong cross-bar. The two vertical screws v on the end of the cross-piece of the roller-holder serve to adjust the rollers in height, while the two horizontal screws h allow one or other side of the roller to be pushed forward. Finally the two horizontal screws H regulate the distance of the rollers from the knifeholder.

The great advantage which the author claims for this microtome is that it can be used especially for relatively soft material. The present instrument allows of a raising of the object-stage 6 cm., so that objects 10 cm. broad, 15 cm. long, and 6 cm. thick, can be cut from beginning to end.

As regards the after treatment of the sections on the paper band, the author has made several improvements on the methods previously employed. In the first place there is no necessity to cover the sections with adhesive mixture before placing them in the benzene bath. It is sufficient to lay the plates carefully between canvas.

The whole series of operations in the use of this microtome are summarized as follows:---

A. Cutting and banding the sections on gummed paper. Numbering.

B. Covering the paraffin layer with a coat of celloidin, a process which involves

1. Placing the plates horizontally between canvas in a benzeneturpentine bath. Several hours.

2. Evaporating and drying.

3. Placing in 95 per cent. alcohol between canvas. 12 hours.

4. Evaporating and drying.

5. Collodionizing by immersing twice in thick collodion.

6. Keeping provisionally in 80 per cent. alcohol or glycerinalcohol.

C. Staining after removing the paper beneath the sections by immersing in water.

D. Clearing, &c.

704

1. Placing in weak alcohol between canvas, and afterwards,

2. With fresh paper beneath, and after drying, in strong alcohol.

3. Immersing in a bath composed of three parts of oil of turpentine and one of creosote, and then in liquid paraffin.

4. Covering with a second layer of paper and arranging in series.

According to the author's experience by far the most satisfactory adhesive material for sticking the sections on the paper-band is a thick solution of gum arabic.

Freezing Microtome.—We append a figure of Dr. T. Taylor's microtome, a description of which has already appeared.\* Fig. B shows a sectional view.



New Cup for Sections.<sup>†</sup>—Dr. Eternod, in view of the inconveniences of the ordinary porcelain cups used for collecting series of sections of embryos, has had recourse to the new processes of manufacture adopted by the firm of Leybold in Cologne, by which glass vessels are made by

† Zeitschr. f. Wiss. Mikr., ix. (1892) pp. 13-4.

1892.

3 в

<sup>\*</sup> See ante, p. 565.

# 706 SUMMARY OF CURRENT RESEARCHES RELATING TO

soldering the pieces together by a special cement. The new cup (figs. 86, 87, and 88) made by this firm consists of a very thick glass plate g, ground on one face and pieced with a number of holes (fig. 87, b and fig. 88, k). A sheet of thinner glass i, soldered to the thick plate by cement (fig. 88, h) forms the bottom of the box and converts the holes



in the thick plate into a series of cups; while a third piece of ground glass, fitting accurately on the ground surface of the thick plate, forms the cover.

The apparatus can be placed on a small box d, provided with an inclined mirror e, which illuminates the cups from below so that their contents can be readily recognized.

To remove Oil and Grease from Whetstones.\*—The process consists in stirring up whitening with water and applying it with a brush to the whetstone which has been warmed in an oven.

Oil of Anise-seed as an Imbedding Medium for the Freezing Microtome.<sup>†</sup>—Dr. H. Kühne has discovered that oil of anise-seed may very successfully be used as an imbedding medium for cutting sections with the freezing microtome. The procedure is as follows:—Pieces about 2 mm. thick are placed on blotting-paper to remove the alcohol, and then immersed in a capsule containing the anise-seed oil for 12–24 hours. When thoroughly saturated with the oil—and this is easily recognized by the clearing up of the material—the pieces are placed on the microtome and sectioned. The sections are temporarily transferred to anise oil on a glass rod, and when all the piece is sectioned, are placed in alcohol (twice repeated) to remove the oil. When all the oil is removed, the sections are ready for staining.

Method of Cold-imbedding in Gelatin.<sup>‡</sup>—M. C. Brunotti gives the following formula which he has used for some time with very good results and has found very useful for histological purposes. In 200 grm. of distilled water are dissolved by aid of heat 20 grm. of the

- \* Zeitschr. f. Wiss. Mikr., ix. (1892) p. 135.
- † Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) pp. 28-30.
- ‡ Journ. de Bot., vi. (1892) pp. 194-5.

white commercial gelatin sold in thin flakes. After filtering through fine cloth about 30-40 ccm. of glacial acetic acid and a gramme of perchloride of mercury are added.

The object of these additions is to keep the gelatin liquid and also to preserve it. At the ordinary temperature, 15°, it has the consistence of thick syrup. According to the season and the temperature it is easy to modify these proportions.

The object to be sectioned is placed first of all in this gelatinous solution diluted with twice or thrice its volume of pure water. It is afterwards immersed in the thick gelatin solution, a little of which has been poured into a paper box. The mass is then set by placing the paper box and its contents in a crystallizer and then carefully pouring round it some spirit. If alcohol should be unsuitable other hardening agents such as picric acid, bichromate of potash, &c., may be substituted, but these reagents act more slowly than spirit.

When sufficiently firm the mass may be sectioned in the usual way and the sections mounted in gelatin or glycerin, or be freed from the gelatin imbedding by dissolving it in water.

### (4) Staining and Injecting.

Methylen-blue Staining of Nervous System of Invertebrata.\*-Herr O. Bürger, when investigating the nervous system of Nemertine worms, seems to have obtained very good results, judging at least from the coloured illustrations said to be faithful representations, by injecting these animals with a fluid made by dissolving 0.5 grm. methylenblue in 100 grm. of 1/2 per cent. cooking salt solution, or with a simple watery solution of the same strength. Sca water, in which methylenblue is imperfectly soluble, is quite unsuitable for the purpose. The injections were made frequently, one injection imparting only a faint staining. The best period for injecting was in the half-dead condition, or in that state when the animal or parts thereof still show signs of life. The time required for bringing about good injection results was at least 6-8 hours, and sometimes much longer. After a time the author found that satisfactory results were more easily obtained by merely injecting a part or organ. The preparations were fixed with dilute picrate of ammonia, and afterwards put up in glycerin to which a trace of ammonia had been added.

The staining does not last very long, and hence in examining a specimen it is advisable to begin at those parts from which the blue colour first disappears, viz. at the periphery.

Permanent Preparations by Golgi's Method.<sup>†</sup>--Dr. G. C. Huber finds that permanent preparations of nervous tissue stained by Golgi's method can be obtained in the following manner:--The pieces are to be hardened and silvered according to the procedure advised by Ramon y Cajal and von Kölliker, and celloidin sections cut up under 95 per cent. spirit. The sections are then immersed for 15 minutes in creosote and then transferred for some minutes to turpentine. After this they are

<sup>\*</sup> Mittheil. Zoologisch. Station zu Neapel, x. (1891) pp. 206-54 (2 pls.).

<sup>†</sup> Anat. Anzeig., vii. (1892) pp. 587-9.

# 708 SUMMARY OF CURRENT RESEARCHES RELATING TO

spread out on a slide, and having been mopped up with blotting-paper, are covered with turpentine balsam. The slide is then gradually and very carefully heated over a flame until the balsam has become so inspissated that it sets hard when cooled. At this stage the cover-glass is put on the hot balsam. The heating takes from 3-5 minutes.

Staining Fibrin.\*—M. Sabouraud communicates a method for staining fibrin which is said to be superior to that of Weigert. Pieces of chancre fixed in Müller's fluid are placed for 15-20 hours in the following solution:—Tannin 1-200, alcohol 10 ccm. to 200 of the solution; they are then stained with anilin-violet (Ehrlich) and then coloured by the Gram-Weigert method, in which during decoloration clove oil is substituted for anilin oil.

Some Facts about Lustgarten's Method for Staining Syphilis Bacilli.<sup>†</sup>—M. Sabouraud, after having frequently failed to find bacteria in syphilitic products by Lustgarten's method, lighted on a case of ulcerating gumma, in the pus of which he succeeded in demonstrating by Lustgarten's method some bacilli. These bacilli were not stained by Ehrlich's method. The author therefore presumed that he had found the bacilli of syphilis. But a guinea-pig having been inoculated with this pus, died of tuberculosis.

The author then raises the question whether Lustgarten, who did not make any inoculations on guinea-pigs, may not have been mistaken in the true character of the growths. Indeed, it was found that Lustgarten's method was extremely useful for demonstrating tubercle bacilli, especially in the liver.

The author gives a new method for preparing sulphurous acid solution.

New Method for Finding Tubercle Bacilli in Sputum. ‡ — Herr Dahmen has devised a modification of Biedert's method, the principle of which consists in separating the solid from the liquid portion of the sputum by boiling with caustic soda.

The author states that the same result may be arrived at by heating the sputum for 15 minutes in a vapour bath. The solid particles almost immediately fall to the bottom and, after the liquid portion has been poured off, are well mixed up in a mortar and are ready for examination.

Malachite-green as an Extracting Pigment.§—Malachite-green, says Dr. H. Kühne, when dissolved in anilin oil has the power of extracting fuchsin, methylen-blue, and crystal violet from sections, and specimens of bacteria prepared in this way are excellent for demonstration purposes on account of their sharp differentiation.

For staining tubercle bacilli the method is as follows :—The sections are stained in cold phenol fuchsin for 15 minutes; they are then washed in water and alcohol and afterwards transferred to a saturated solution

\* Annales Inst. Pasteur, 1892, p. 184. See Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) p. 807.

† Annales Inst. Pasteur, 1892, p. 184. See Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) p. 807.

<sup>‡</sup> München. Med. Wochenschr., 1891, No. 38. See Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) pp. 41-2.

§ Centralbl. f. Bakteriol. u. Parasitenk., xi. (1892) pp. 756-8.

of malachite-green in anilin oil. Very thin sections are decolorized in 2-3 minutes; thicker ones require a correspondingly longer time. The sections are then placed in turpentine for a short time (they should be of a delicate blue-green hue) and then in xylol to remove the turpentine, after this they are ready to be mounted in balsam.

Staining of other bacteria by the malachite-green method is done as follows:—(1) Stain sections in phenol fuchsin for 5 minutes. (2) Wash in water and then just immerse in spirit. (3) Remove to pure anilin oil until quite cleared up. (4) Wash out the anilin oil in turpentine (about 1 minute). (5) Transfer to more or less strong malachite-green anilin oil for 10 minutes or longer, according to thickness of section. (6) Immerse the section in turpentine, xylol, balsam.

It may be mentioned that when sections are placed in turpentine they become covered with precipitated pigment. This soon disappears, and if the sections be left in too long the malachite-green is completely extracted. It is, however, impossible to give an approximate notion of the time required for treating with turpentine. If, however, the green be too much extracted, the sections may of course be returned to the malachite-oil.

The staining of the bacteria is said to be quite undisturbed by this treatment.

Double and Metallic Stains.<sup>\*</sup>—Prof. A. B. Aubert has prepared the following useful compilation.

Bismarck Brown and Methyl-Green.—Bismarck brown, a concentrated warm aqueous solution, or a weak alcoholic solution. Methyl-green, 0.5; water, 100. Sections to be kept in about 15 minutes; wash with water, stain dark green, wash, soaking in alcohol until grass-green; clear in oil of bergamot, xylol; mount in balsam.

Borax-Carmine and Indigo-Carmine.—1. Carmine, 2 grm.; borax, 8 grm.; water, 130 grm. 2. Indigo-carmine, 8 grm.; borax, 8 grm.; water, 130 grm. Mix 1 vol. of 1 and 2. Sections are taken from alcohol and stained in from 15 to 20 minutes; transfer from 15 to 20 minutes to concentrated solution of oxalic acid; wash; dehydrate; mount in balsam.

*Eosin and Methyl-Green.*—I. Eosin, 1 part; methyl-green, 60 parts; alcohol, 30 per cent.; warm. II. A. Eosin, 1 part; water, 50; alcohol, 50. B. Methyl-green, 1; water, 100. Sections stain in I. in 5 or 10 minutes; quickly wash in successive alcohols, mount in balsam or glycerin. II. is for blood-corpuscles; dry the blood (thin layer) on the slide; treat with A 3 or 4 minutes, wash stain off with water; treat with B 2 or 3 minutes, wash, dry, mount in balsam (red corpuscles red, nuclei and leucocytes bluish-green).

Fuchsin and Methylin-Blue.—A. Fuchsin solution in alcohol, 8 to 10 drops; water, a watchglass-ful. B. Concentrated aqueous solution of methylin-blue. Tissues to be hardened in chromic acid, &c.; stained in A for several to 24 hours; washed with alcohol, stained in B for 4 to 5 minutes (nuclei red, tissues blue).

Hæmatoxylin and Eosin.—Solution of eosin in glycerin in which some salt has been dissolved; concentrated solution of alum in glycerin

\* The Microscope, xii. (1892) pp. 152 and 3.

and glycerin hæmatoxylin. Stained specimens washed in alcohol containing a little eosin; clear in oil of cloves; mount in balsam. *Picrocarmine.*—I. Carmine, 1 grm.; liquor ammoniæ, 4 ccm.; mix

*Picrocarmine.*—I. Carmine, 1 grm.; liquor ammoniæ, 4 ccm.; mix and add 5 grm. picric acid. II. Carmine, 15 grm.; picric acid, concentrated solution. Agitate the mixture (I.) from time to time for 2 days; let it settle; decant and evaporate decanted liquid at ordinary temperature; redissolve the dry residue in water, making 1 per cent. or 2 per cent. solution; filter when necessary.

II. Triturate the carmine in a mortar until very fine; add enough ammonia to dissolve the carmine; to this add slowly concentrated solution of pieric acid until the mixture has a blood-red colour; keep in a shallow dish until all odour of ammonia has disappeared; filter; keep in a well-stoppered bottle; add a few drops of carbolic acid; filter before using.

Picrocarmine and Eosin.—Solution of picric carmine, 1 per cent., 1 part; watery solution of eosin, 2 per cent., 1 part. For small organisms, 1/2 to 4 days; wash in 70 per cent., then in 90 per cent. alcohol.

Metallic Stains :---

Ammoniacal Nitrate of Silver.—Silver nitrate, 0.75 to 0.5 per cent. with ammonia. Add to nitrate of silver solution enough ammonia to redissolve the precipitate which forms at first; dilute to 0.75 per cent. or 0.5 per cent.

Gold Chloride.—A. Gold chloride, 1 grm.; water, 2000 ccm.; hydrochloric acid, 30 drops. B. Alcohol, 1 part; formic acid, 1 part. Sections treated with A, then transferred to B, where reduction of the gold takes place.

Potassium Gold Chloride.—A. Potassium gold chloride, 1 part; water, 10,000 parts; hydrochloric acid, a trace. B. Water, 2 to 3000 parts; hydrochloric acid, 1 part. C. Alcohol, 60 per cent., 1000 parts; hydrochloric acid, 1 part. Sections hardened in ammonium bichrowate are put in A for from 10 to 12 hours, until they are of a light violet colour, washed in B, and transferred to C.

Osmic Acid and Silver Nitrate.—A. Potassium bichromate, 2 per cent., 10 parts; osmic acid, 1 per cent., 1 part. B. Silver nitrate, 1 part; water, 200 parts. Objects soaked in A for several hours, transferred to B for at least 8 hours.

Silver Nitrate.—A. Silver nitrate, 0.25 to 0.5 parts; water, 100 parts. B. Salt, 0.75 parts; water, 100 parts. Sections 20 to 40 seconds in A, then in B; move them about in both, then expose to the light.

Silver Nitrate and Silver Iodide.—A. Silver nitrate, 1 part; water, 100 parts. B. Silver iodide, 1 part; water, 100 parts; potassium iodide, a trace. C. Silver nitrate, 0.1 part; water, 100 parts. Put the sections in A (in the dark); after 2 or 3 minutes add a few drops of B; wash the sections in water and expose for 2 days in C to the action of the light (cornea.)

#### (6) Miscellaneous.

Wethered's Medical Microscopy.\*—This little work, one of the numerous guides for the much-written-for medical student, is a good example of the compilations so frequently seen of late years. Its chief distinction is its title, which is inviting and alliterative. In the letterpress there is nothing particularly new except, perhaps, some spelling variations such as Schyzomycetes, Leucaemia, Jolgi; but one original statement deserves a passing notice: the diameter of amyloid casts varies from almost one inch to not more than that of a red corpuscle. This is quite new. Casts of this larger size would not probably require the aid of a Microscope for their discovery, and might be fished up with a walking-stick. The work is apparently quite a compilation.

Reactions of Callus and Paracallus.<sup>†</sup>—Mr. S. Le M. Moore gives the following microchemical reactions for true callus, and for the proteid substance which he distinguishes from it, under the name of paracallus.

Callus rapidly dissolves on warming sections in Millon's fluid, and displays no tendency to become red. It is soluble in boiling nitric acid; hence the xanthoproteic test does not succeed. On running in caustic potash after sections have lain some time in copper sulphate, callus swells up, but does not turn blue or pink. After a good soaking in syrup, sulphuric acid swells callus so that it is almost invisible but it never assumes the slightest tint of pink. After many experiments with a peptic fluid, allowed to act as long as 86 hours, callus undergoes not the least change in form or general appearance; and it now reacts quite normally with picric blue and corallin soda. The same result followed every attempt to dissolve callus in a pancreatic fluid (Fairchild's pancreatic extract).

Paracallus, on the other hand, stains yellow with pieric blue; takes a temporary pink with corallin soda; does not swell up appreciably in sulphuric acid or caustic potash: is stained brown by iodide; is not acted on by carmine; and gives good proteid reactions. It frequently dissolves in a peptic as well as in a pancreatic fluid.

\* London, 1892, crown 8vo, 412 pp., with illustrations.

† Journ. Linn. Soc. (Bot.), xxix. (1892) p. 232. Cf. suprà, p. 630.

# MICROSCOPY.

a. Instruments, Accessories, &c.\*

(1.) Stands.

Beck's Improved "Continental" Model Microscopes.\*-This is a new and very solid and substantial model Microscope designed by



\* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous.



Messrs. R. and J. Beck specially for class work—on the lines of the foreign models, but made with a steadier foot, a larger stage, and mirror with a vertical adjustment.

856



It is made in three forms :--Fig. 89, Sliding coarse-adjustment; fig. 90, rack-and-pinion coarse-adjustment; fig. 91, with swinging and focusing substage carrying Abbe condenser and iris diaphragm. 3 м 1892.

FIG. 91.

# SUMMARY OF CURRENT RESEARCHES RELATING TO

Nachet Microscope.—The large model No. 2, represented in fig. 92, is very solidly built, and possesses all the requirements necessary for microscopic work of all kinds. It can be inclined to the horizontal.



The stage rotates about the optic axis, and carries the movable slideholder. The coarse-adjustment is by rack and pinion, and the fine-by the new system of micrometer-screw,<sup>\*</sup> with divided head indicating the 1/400 part of a mm. The double plane and concave mirror is mounted on joints. The draw-tube is divided into millimetres.

The whole illuminating system, consisting of a wide-angled Abbe condenser (N.A. 1.40) with iris diaphragm, is raised or lowered by the screw V. The iris diaphragm is mounted on the wheel R, worked by

the tangent screw T, which by a very slight movement causes the aperture of the diaphragm to pass from the centre to the periphery of the condenser. The handle I serves to open and close the diaphragm.

Fine-Adjustment of the Beck Pathological Microscope +--- The fine-adjustment of Beck's new Pathological Microscope is stated to be one of the most sensitive and delicate fine-adjustments yet produced. It is constructed as shown in the accompanying figure. The body of the instrument is supported upon the barrel DD; this barrel is accurately and smoothly fitted to the tri-At the top of the angular core E E. barrel DD is screwed the cap G, to which is attached the rod C. This rod passes through the centre of the core E E, and connects with the lever arm A at B. The action of the spring J which is wrapped spirally around the rod C raises the body of the Microscope and holds the lever arm A tightly against the screw arm F. The slightest motion, therefore, of the screw F is communicated through the lever A and the rod C to the body of the Microscope.

The great delicacy of this arrangement will be appreciated when it is noticed that the distance from I H is double the distance I B, therefore any motion at B is only half that at H. This adjustment is one of the most delicate made for use with high powers.

### (2) Eye-pieces and Objectives.

A Recent Improvement in the Microscope.<sup>+</sup>—"L. H." writes as follows:—"I purchased some time back a valuable Microscope, which cost over 201. with accessories, but I was not satisfied with it, and neither have I ever been satisfied with those instruments exhibited at London microscopal *soirées* at Morley Hall, Hackney, although of celebrated manufacturers. The Zeiss lenses, also, have not come up to

\* See this Journal, 1886, p. 837.
‡ Engl. Mech., lvi. (1892) p. 17.

† Microscope, xii. (1892) pp. 183-4.

3 м 2

eck Pathoine-adjustcal Microthe most ljustments tructed as ure. The supported el is accuo the triop of the cap G, to This rod the core ver arm A g J which the rod C scope and y against st motion, communithe rod C

FIG. 93.

my standard of excellence, or liking. What I wanted to attain to was simply this: to be able to look through the tube as though there were no glass or medium in it; for it to be absolutely transparent or translucid, absolute distinctness of detail, edges of objects absolutely sharp and defined, the same with bubbles of air in mounts, to be able to see the surface of the finest polished glass-films with any power, and to see the depth of an object at the same focus. After many experiments with diaphragms, at last I said to myself; achromatic lenses are made of flint and crown-glass; the refractive index of flint is 0.026; its dispersive power, 0.052; some specimens refractive power of 0.029, dispersive power, 0.048; that of crown is 0.038 dispersive power. 0.018 refractive power. The refractive index of Canadian balsam is 0.021, dispersive power, 0.045. The refractive power of castor-oil, 0.018; dispersive power, 0.036. After reckoning up the probable results of gain from these indexes, I concluded that after opticians have corrected the colour and achromatized, or made achromatic their lenses, they required to be further balanced and more nicely balanced than they have been in regard to their refractive and dispersive power. On experimenting, I found that in the Microscope a material element was the place of its being used, and that although Canadian balsam was more whitish to the eye, it dispersed nine times and refracted three times too much to make an even balance. It does not give good results when used on object-glass in any way. On carefully cleaning with spirits of wine an achromatic lens of the same size as the body of the Microscope-tube, and cementing them together with castor-oil and fixing them at the end of draw-tube, judge of my delighted surprise when I found I had thereby attained more than everything I wished for with my Microscope, and equally attained it whether I used 2-in., 1-in., 3/4-in., 1/2-in., 1/4-in., 1/8-in., or 1/25-in. object-glass. The tube of the Microscope was absolutely translucent, and I could look through it as if it contained no glasses whatever. Field of view absolutely flat; focus of lens at end of draw-tube 81 in.; full length of Microscope-tube a little over 1 foot. Castor-oil as an immersion does not act properly. On testing Canadian balsam with a telescope of 2 feet and 13-in. objectglass, and comparing it with the object-glass cemented with castor-oil, the latter gave incomparably best results. I can see the flies playing in the air between the line of sight and the buildings or conservatories in the distance."

On this we need make but few remarks, for, as is well known, the action of a lens at the end of the draw-tube has been advocated and triel *ad nauseam*. Dr. Piggott's "Aplanatic Searcher" is practically one of the instances; it was about to revolutionize the practical optics of the world, only unfortunately it failed and is nowhere to be found to-day.

It is possible that the "8½-in. lens" at the bottom of the draw-tube may, by reducing the power of the optical combinations used, slightly increase the light and so produce what by contrast is a sharper picture, but a mere change to a lower eye-piece would have done the same.

The refractive indices appear to be wrong; but, neglecting this, the film cementing two lenses together is of necessity very thin and has parallel curves; therefore assuming that the writer *is* correct in his

860

statement about Canada balsam and its refractive index (which is against all that the best authorities have discovered, viz. that the refractive index of Canada balsam is  $\mu_{\rm D}$  1.540) involving a dispersion of nine times and a refraction of three times too much for optical balance when compared with castor-oil, yet the effect of a *thin* film with parallel surface cannot be sufficient to make *any* perceptible difference.

Castor-oil, moreover, crystallizes, and is practically unsuitable for a permanent cementing medium.

## (3) Illuminating and other Apparatus.

Standard Glass and Speculum Metal Centimetres.\*—Dr. M. D. Ewell writes:—"I have ruled two centimetres on speculum metal subdivided into millimetres, the first millimetre into 1/10, and the first 1/10 mm. into 1/100 mm. These were sent to Prof. William A. Rogers several months since for investigation as to total length.

The five glass centimetres similarly subdivided, which were made the subject of a communication at the last meeting, have been in my possession the last three or four months, but are now in the possession of the Treasurer. My experience with glass micrometers has been such as to lead me to delay their investigation till sufficient time has elapsed to ensure their durability.

The lines on the five centimetres above referred to are still in as good condition as when made, and bid fair to be permanent.

The lines on the Fasoldt centimetre No. 2 (glass), upon which so much time was spent some years since by myself in comparing it with 'Centimetre A,' have so deteriorated as to make it entirely worthless for micrometric purposes, notwithstanding they are covered, and therefore not exposed to the deteriorating influences operating upon ordinary glass micrometers. Owing to the uncertain life of such scales, it has been thought wise not to bestow so much labour upon the five glass centimetres above referred to till more time has elapsed. I have, however, in order that they may be available for present use, compared the first 1/10 mm. of each of said scales with the first 1/10 mm. of 'A,' and deduced the following provisional corrections, which are the mean of ten measurements of each space.

The comparisons, of which the following are the results, were made by means of a Bulloch Professional stand No. 2, a Zentmayer filar micrometer, and a Bausch and Lomb one-half opaque illuminator, with daylight received from the right of the instrument :—

Sc	Scale.			rectio	n to F Umm	'irst	Correction to First 1/5 mm.
Ewell ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,,	•••	I. II. III. IV. V.	- + + +	••	••• •• ••	$ \begin{array}{c} 0.5 \\ 0.0 \\ 0.4 \\ 0.2 \\ 0.5 \end{array} $	Not determined. + 0.4. Not determined.

\* Proc. Amer. Soc. Micr., xiii. (1891) pp. 71-2.

The two speculum-metal centimetres above referred to, with their investigation as made by Prof. Rogers, will subserve the purpose of substandards, and the five glass centimetres can be used by observers for the verification of their glass stage micrometers, so that there need be hereafter no occasion to use 'Centimetre A,' except as a final standard of reference. I would therefore respectfully recommend that 'Centimetre A' be deposited with the Superintendent of the United States Coast and Geodetic Survey, and that hereafter the two speculum centimetres be the *working standard* of the American Society of Microscopists."

Revolving Stage for Viewing Microscopic Sections, &c.\*-Dr. T. Taylor, the chief of the Division of Microscopy in the U.S. Department of Agriculture, has devised a stage of which he gives the following account:--

"This plate exhibits a view of a new and improved form of revolving brass plate which I have recently devised in order to supply a need long felt in the division. It may be attached to any Microscope, and is designed principally for reviewing and comparing serial sections and textile fibres. This revolving plate is pivoted upon the substage by means of a downward-projecting pin. It may thus be rotated freely at the pleasure of the operator. Slides mounted with subjects for investigation and comparison are secured by means of spring clips upon the surface of the plate.

A stage of this description which I am accustomed to use exhibits eleven different samples of wools. In jury trials relating to wools I have found it sometimes desirable to have six Microscopes in use at one time in illustrating the respective characteristics of various samples of wool. Even with this number the parties are seldom satisfied, as one person is obliged to move from one instrument to another, interfering, perhaps, with the view of other observers. The system I have initiated saves much time—an important consideration in the court-room. By means of the revolving plate eleven diverse samples may be compared in less time than an observer could move from one Microscope to another.

Six stands of this model were on exhibition at the fourteenth annual meeting of the American Microscopical Society, recently held in this city, and the invention gave universal satisfaction. The publishing committee of the society have requested a description of this plate for the forthcoming volume of Proceedings.

I use a similar form for high powers, consisting of perfectly clear glass 2 mm. in thickness, circular in form, like the preceding, and, like it, attachable to the plane stage of a Microscope. On this plate the objects may be arranged upon its margin, the same as on the usual glass slides, and the cover-glass fixed upon them, thus dispensing with clips, which interfere somewhat with the objective when using high powers. Or the plate may be perforated, as in the metal plate, the mounts fixed by means of wax or a drop of paraffin at the edges of the slides. This method, I find, renders the object sufficiently steady for examination, and the wax has the advantage of being easily removed when it has answered the purpose, leaving a clean plate for change of subject or

\* Report of The Microscopist for 1891, pp. 413-4.

for further investigation. The diameter of the revolving plate is only limited by the construction of the Microscope-stand, to which it is an adjunct."



Heating Apparatus for Crystallographic Optical Work.\* — Herr R. Fuess describes three forms of heating apparatus which serve for the optical examination of crystals at high temperatures.

The first apparatus, represented in fig. 95, is intended for temperatures up to  $450^\circ$ , which can be measured by a mercury thermometer. It consists of a box formed of a bent brass tube, of rectangular section, through which a stream of hot air is led. The arm A' which rests upon the stage of the Microscope serves as air-bath for the crystal under examination, while the other, directed upwards, acts as a chimney through which the heated gases escape.

\* Neues Jahrb. f. Min., Beilage Bd. vii. (1890) pp. 406-16.

# 864 SUMMARY OF CURRENT RESEARCHES RELATING TO

Near the bend at b the tube is cut through; and in the circular apertures fit glass plates which give passage to the light, and serve to protect the objective and condenser. Between these plates in the middle of the box is the crystal holder which consists of a small strip of glass resting on two supports. The whole box is coated with asbestos in order



to reduce the radiation, as well as to protect the hands of the observer from contact with the hot metal. For the further protection of the Microscope, the plate P, which rests upon the stage, is coated with asbestos on its under side, and is connected with the heating apparatus by four glass feet. In the case of large instruments with movable stages, the apparatus is fastened to the stage by the binding screw S.

The heating of the crystal is effected by a Bunsen burner g g', the tube of which cuts through the inclined arm of the box, and lies with the greater portion of its length in the horizontal part. At g'it is bent at a right angle, and the part opposite the opening of the box is slit for the exit of the gas, which burns with a broad blue flame.

The position of the burner can be adjusted so that the flame approaches or separates from the object. By this means a very precise regulation of the temperature can be effected.

At the bend of the burner a tube r can be inserted which serves for the introduction into the box of water vapour or cold air. For this purpose the two branches of the tube can be connected respectively with a flask of boiling water and with a bellows. By the latter a rapid cooling can be effected, or a blowpipe flame produced when a tube with circular opening is fitted over the ordinary slit of the burner.

The reservoir of the thermometer is in the shape of a horse-shoe, the two arms of which rest on the object-holder, and enclose a space of about 7 mm. square which is occupied by the crystal, so that a uniform heating of thermometer and crystal is assured.

The second apparatus, represented in fig. 96, is for temperatures up to a red-glow. The source of heat is an electric current passing through two pieces of platinum foil. On a rectangular stage of slate, with wide central aperture, are screwed two brass plates P P', isolated from each other. Attached to the plate P' is a small bracket l, the wedge-shaped pointed end of which projects slightly over the edge of the central aperture of the stage, and carries a small Fig. 96.

A second similar bracket l' can be screwed over the first by the screw r, so that the pin of l fits into a corresponding hole in l'.

Opposite the clamp thus formed by l and l', is a similar one L L', carried by the plate P. This clamp, however, is not rigidly fixed like the first, but can be made to approach it to a certain extent by pressing on the end against a spring attached to the under side of P.

The two clamps serve to stretch between them the two pieces of platinum foil B, which are pieced in the centre with small apertures. After removal of

with small apertures. After removal of the upper parts L' l', the clamps are brought closer together by pressing against the spring, and the two pieces of foil are placed upon the lower parts L l, so that the projecting pins pass through corresponding holes in the foil. The upper parts are then put on, and the pressure on the spring is released, so that the pieces of foil are firmly stretched, and form a metallic connection between the plates P and P'. The crystal plate is inserted between the pieces of foil and the pressure due to the spring suffices to hold it firmly in position, even when the Microscope is directed horizontally. For greater security, however, the lower piece of foil is provided with a small projecting rim. The apparatus is attached to the Microscope-stage by the ordinary clips, which press upon the pins ss' projecting from the plate of slate. The electric current is brought by the wires KK, which are screwed to the plates P, P'. In the Berlin Mineralogical Museum, the current is supplied by a Raub thermo-battery, which produces a current of 15 ampères, with electromotive force of 3 volts. A rheostat is used for the regulation of the temperature.

For observation with convergent polarized light at high temperatures, the lens fastenings of objective and condenser must be protected with capsules of horn or paper; but even with this precaution a close approach of condenser and objective can only be allowed for a very short time.

The apparatus is, therefore, not well adapted for observation between crossed nicols in convergent light, and can only be used with advantage for small crystals.

The third apparatus, heated by gas, for observation with parallel and convergent light at high temperatures, is intended to remedy these defects. It differs from the two just described in so much that it is not connected with the stage of the Microscope, and so cannot be rotated with it. It is therefore most conveniently used with a Microscope such as that of Dick and Swift, in which the two nicols can be



# 866 SUMMARY OF CURRENT RESEARCHES RELATING TO

simultaneously rotated while the stage remains fixed. The apparatus, which is represented in fig. 97, is carried by a support T attached by the screw s to the fine-adjustment column of the Microscope. On the end of the support rises a hollow column S, terminated by a hollow axis which carries the burner B B'. The latter can be brought into the vertical position, which is shown dotted in the figure, by means of the handle g, and it is always in this position that the crystal is heated. The gas



brought by the tube G passes through the cock H into the column, and thence through the axis to the burner B. At the foot of the column are holes for the admittance of air, which can be regulated by means of a second cock h'. The arrangement of the crystal holder is somewhat similar to that adopted in the second apparatus. As in that arrangement the crystal is held between two pieces of platinum foil, the middle portions of which form square plates, pieced by central apertures. From the square central part of one of these plates there project on each side two arms, the ends of which are riveted to the under surface of the steel rods o'. From the middle part of the second plate two arms project between the arms of the first plate, and are riveted to the upper surface of the steel rods. The arms of the two plates are thus separated by the thickness of the rods; and when the latter are driven apart by the effect of the spiral spring P, the central parts of the plates are pressed together and firmly clamp the crystal placed between them. The opening of the clamp is effected by means of the screw q, which presses upon the The crystal is oriented by means of rod o', jointed to the plate n. the horizontal displacement of the holder, and its rotation about the axis at the head of the column, which is regulated by the screw v. After the orientation of the crystal, and adjustment of the objective and condenser, the burner is brought into the vertical position, and the crystal is heated. It is then quickly turned back again, and by means of the stop of the screw v the crystal keeps its former orientation. On turning back the burner, the flame is at the same time extinguished by the rod A acting upon the cock H, and cutting off the gas supply. When the burner is brought back again to the vertical position the cock is again opened, and the burner re-lighted by means of a small gas flame, fed by the tube t, which is kept constantly burning. In the burner is a small tube u, communicating with a bellows, which serves either for the production of a blow-pipe flame, or for the cooling of the apparatus.

The Reflector with the Projection Microscope.\* — Mr. G. B. Buckton urges the advantages of a catoptric as compared with a dioptric arrangement in the following letter :—

"The lantern is now used for so many purposes—scientific, photographic, and recreative—that any improvement in its construction will be acceptable. When we look into this instrument whilst at work we must be disappointed at the large quantity of light lost by reflection and by dispersion—light which ought to go to the illumination of the screen. In the ordinary form of the lantern three lenses of dense glass are employed as condensers. Each of these six surfaces reflects and scatters the light, and the glass itself is absorbent of its rays.

The dioptric construction of the projection lantern has been well worked out by Messrs. Wright, Newton, Salomons, and others, but the catoptric principle, which would eliminate almost entirely these disadvantages, has been scarcely at all studied.

Although my experiments have been made solely with the limelight in various forms, the following remarks may equally apply to light given by the electric are :--If a reflector be used instead of the ordinary condenser, it is obvious that the position of the lime cylinder must be reversed. This will present no difficulty, for the tube holding the jet can be bent into a helical form. The dark image of the lime cylinder also will have no more practical disadvantage than is experienced by a like image formed by the small plane speculum of the Newtonian telescope.

As to the mirror itself, although a parabolic form is the most correct, a spherical surface will be sufficient for mere illuminating purposes, and thus expense may be spared in the grinding of the more difficult curve. A speculum of from 5 to 7 in. diameter, having a radial curvation of from  $2\frac{1}{2}$  to 3 in., will grasp a large quantity of light, much more than that obtainable from the 5-in. condenser usually employed.

Silver deposited by one of the various reducing processes on the

surface of a clear glass lens will have many advantages over a metal mirror. The front surface will give, perhaps, the finest definition, but by silvering the back part of a spherical glass film, or that of a ground lens, the brilliant portion will remain untarnished for an indefinite time, and the whitish bloom formed by slow volatilization of the incandescent lime is easily removed. This silver film adheres with remarkable tenacity, and it will bear a great deal of heat without blistering or becoming detached. I have had considerable success in constructing such mirrors from the large ornamental glass spheres blown in Germany, and silvered within by Liebig's process, viz. with milk, sugar, and ammonio-nitrate of silver.

A glass sphere of 10 or 11 in. in diameter may be easily cut into eight or nine mirrors by a red-hot iron, and this without disturbing the silvering, which will require only gentle friction with a pad of cotton impregnated with a trifle of rouge to brighten it. Thus, at the cost of a few shillings, eight or more mirrors can be made, and also provision be made against possible accidents of cracking by heat.

The light-radiant is so placed that the secondary focus is intercepted by a plano-concave lens of dense glass, as has been happily proposed by Mr. L. Wright. The convergent rays from the speculum are thus made into a parallel beam, which must be deprived of its heat by an alum trough, for the light and heat at the substage condenser are very great.

Convergence, I find, is usefully promoted by a plano-convex lens of about 8 in. focus placed two or three inches before the above-noted plano-concave lens. In all other respects the arrangements are like those of the usual modern projection Microscope.

I have pretty constantly used the ether-oxygen saturator, and I consider it to be perfectly safe if ordinary precautions be taken. The oxygen, compressed in cylinders, is much recommended, as there can be no mixture of vapour, except at the right place. The U-shaped horizontal saturator, plugged with flannel, must be well charged with ether, or with the best gasoline, and care should be taken, before beginning or ending an exhibition, to shut off the oxygen-tap before closing the ether-tap. This will prevent the harmless 'snap' from the mixture in the small chamber at the joining of the gas-tubes. If a disc more than 8 ft. be required for the Microscope, it will be well to use hydrogen gas instead of ether, since the calibre of the jet cannot in the ether light very well exceed 1/14 in. As an extra security I pack the mixing chamber with asbestos fibre moistened with glycerin; but, as before urged, the oxygen must leave the saturator saturated.

To ensure the coincidence of the foci of the reflector with the optical axis of the Microscope it will be well to place three adjusting screws in a triangle behind the mirror, and this last may have both a small vertical and horizontal movement. I claim for this catoptric arrangement a larger grasp of light than can be got from ordinary lenses, and this may be effected also at a small outlay. For the amateur constructor the plan will afford many advantages."

### (4) Photomicrography.

**Processes of Photomicrography.**—The following is the text of Dr. Piffard's letter, read at the October Meeting:—"In the last issue

868

(April) of the Journal, Mr. Gifford, in connection with the resolution of the Amphipleura, advances an idea which is probably novel to many, namely, photomicrography by means of monochromatic yellow light. As I have personally worked in this direction for some time, and have carried the idea a step farther than Mr. Gifford appears to have done, I take the liberty of bespeaking the courtesy of your pages. I will briefly relate the main facts which led me to the processes I now employ in photomicrography, and which in low power work give me results vastly better than those at present in vogue, although in high power work I have not as yet observed the same advantages.

In 1873 I purchased a No. 4 Hartnack objective (about 1/2 in.). It was a glass of quite moderate angle but otherwise good of its kind. Employed in photomicrography, using the then customary collodioiodide wet plate, the results were not satisfactory. About 1878 I purchased a Wales 4/10-in. Ang. Ap. 90°. This lens was and is still a remarkably good glass to look through, but it utterly failed to give a satisfactory result when used photomicrographically at the time of purchase. Five years ago I tried it again, using the now customary and ordinary gelatin-bromide dry plate. The results were disappointing. About four years ago I became interested in the so-called orthochromatic plates, and as they were not at that time an article of commerce in this country, was obliged to prepare my own, and succeeded in preparing such as were equally sensitive to blue, green, and yellow, and almost wholly insensitive to violet and ultra-violet. I also made such as were remarkably sensitive to yellow, but hardly at all to the other colours. The methods employed were published by me a few years ago in 'Anthony's Photographic Bulletin.' The commercial plates now obtainable in this country are markedly sensible to yellow, less so to blue, and still less so to green. On the whole they are of better quality than similar plates of English manufacture that I have tried, though not equal to some Vogel-Obernetter-Perutz plates that I once had the opportunity of using.

About ten months ago I asked Mr. W. Wales to make me some low power objectives specially corrected for photomicrography. He did so, but the lenses did not satisfy me, and I retained but one of them. I subsequently asked him to make me a 1/4 in. When he brought the lens he said, 'I have put this in a rough mounting in order that you may look through it as it is before I finish its correction for photography, as I shall undoubtedly impair its visual performance to a certain extent. He further said, 'I think it is the best 1/4 in. of its angle (75°) I have ever made.' On visual examination (Podura) the lens left little to be desired, but being curious to see just how far 'off' it was photographically I made a negative using a commercial (isochromatic) plate. The result was a gratifying surprise. On reporting the matter to Wales he said, 'I do not understand it, the lens in the condition in which I gave it to you ought not to have photographed so well.' For comparison I photographed the same scale with a Powell-Lealand 1/4-in. waterimmersion N.A. 1.26 which I have owned for several years. The resulting negative was not equal to that made with the lower angle Wales.

I believe it is generally maintained, and I think with justice, that

the Zeiss apochromatics are not visually superior to first class achromatics of some other makers; but it is also admitted that they have usually given better results photographically.

A few months since I made a critical examination of the colour corrections of my Zeiss 2-mm. apochromatic and I found it slightly overcorrected. The same was true of two other Zeiss apo's. My Wales 1/4 in, was also over-corrected. Reflecting on the facts herein narrated I was led to the provisional induction that to get the best results with orthochromatic plates the lenses should be over-, rather than undercorrected.

To confirm or refute this induction I next tested the 4/10-in. Wales and No. 4 Hartnack, both over-corrected, and found that their performance was much better with orthochromatic than with plain plates. There was, however, something still lacking; the negatives did not possess the absolute sharpness that was desired. The defect in definition especially noticeable at the margins of the picture I attributed to unequal magnification by rays of different refrangibility. To overcome this I excluded the blue, violet, and ultra-violet rays by means of a suitable The one that proved the most satisfactory was a solution of ray-filter. tropæolin (Grubler's 000). This permits the passage of the red, orange, yellow, and a portion of the green. The advantages gained by its use were great, but still were not equal to theoretic demands. To carry out these practically to the fullest extent will require an absolute harmony in illumination, in lens, and in plate, each being adjusted so far as possible to the rays of the same refraugibility. In former times the photographic plates then in use were sensitive only to the G, H, and ultra H regions of the spectrum, and lenses were under-corrected in order to bring the more refrangible actinic rays into coincidence with the more powerful visual ones from the D region.

Insomuch as the present orthochromatic plates are capable of giving results utterly unattainable on plain plates, I am satisfied by practical experience as well as theoretically, that the photomicrography of the future will advance considerably over that of the past if the D region be selected as the standard for illumination, for the sensitiveness of the plate, and for the correction of the objective. The first may be secured by suitable absorptive solutions or the employment of monochromatic yellow light obtained by prismatic or diffraction dispersion. Plates specially sensitive to D light are readily accessible, and there is therefore no difficulty in fulfilling the second indication. The third requirement will necessitate a slight modification of the formulas usually employed by the leading opticians. Mr. Herbert R. Spencer, son and successor of the late Chas. A. Spencer, is now making for me a 1/6 homogeneous immersion specially corrected to the end in view."

Nachet Photomicrographic Apparatus.—The instrument shown in fig. 98 consists of the large model inverted, with camera attached to the side-tube. The distance between objective and eye-piece amounts to  $1\cdot 20$  m. The mirror is silvered, so that the loss of light is insignificant, and the highest objectives can be used.

By reason of its absolute stability and the facility which it offers for very oblique illumination, this instrument is particularly adapted for photographing diatoms. The focal adjustment and arrangement of the illumination can be made with the greatest ease during the examination of the image. For preliminary observations the camera can be easily replaced by the eye-piece.



The latest form of the apparatus for the instantaneous photography of moving objects is shown in fig. 99. It consists of a camera mounted on two supports which slide on columns attached to the base of the apparatus. The camera is connected below with a Microscope of special construction, which enables the observer to examine



FIG. 99.

the objects while the sensitive plate in the shutter is ready to receive the photographic impression. The details of the arrangement have already been described in this Journal, 1886, p. 842.

In front of the Microscope is a stand on which are mounted a mirror, a condensing lens, and an iris diaphragm, which serve for the proper regulation of the light.

Microscopical Illustrations.\*-Mr. H. L. Tolman writes:-" One of the most difficult features in connection with the illustration of scientific articles is the reproduction of the photomicrographs or cameralucida drawings. If the object contains a large amount of detail, a photograph will be the only way by which all the minutiæ can be preserved, but no woodcut can entirely reproduce the original. Of course, it is not always necessary that everything which is seen under the Microscope should be seen in the book illustration, and just here is the point where authorities differ on the requisites of a good woodcut or engraving. Some hold that only the salient parts of an object need be represented; in fact, that the picture is better for having omitted from it all but the few leading features, to which the writer desires to call attention. Others claim that the picture should represent just what the eye sees under the Microscope, free from any of the possible or intentional errors of the artist. There is, undoubtedly, much to be said on both sides of the question.

Those who have studied the astonishing cuts called "diagrammatic representations," the counterpart of which they vainly search for in nature, will be strongly in favour of any method which reproduces an object so that it can be recognized, and the tendency of the art of the present day seems to be in this direction. Fortunately, with this demand comes an improvement in the manner of reproducing photographs and drawings of every kind, which deserves a somewhat ex-tended notice. This is by what is known as the half-tone process, which consists of a photographic copy of the original on a zinc or copper plate and then etching the plate until the drawing appears in relief, and is printed from like an electrotype. In order to convert the smooth, soft shades of a photograph into a form which will prevent them from printing a solid black, they are broken up into a series of dots more or less close, by the interposition of a finely ruled screen in the camera just in front of the dry plate. On the character of this screen depends in great measure the quality of the finished picture, and the skill of the process worker is best shown by a proper selection of the screen to illustrate the landscape or portrait which is to be reproduced.

For bold subjects a coarse screen is appropriate, while for those with great detail and delicate graduations of shades a fine screen is required. These screens are either ruled on paper and copied by the wet plate process on glass or directly on glass, the lines varying in distance from 1/100 to 1/150 in. Obviously, for nearly all microscopical photographs the fine screens must be used, but it will be found that some of the contrast must be sacrificed thereby. Of course, there are some subjects which cannot be reproduced by the half-tone

\* Amer. Mon. Micr. Journ., xiii. (1892) pp. 155-7.

1892.

3 N

## 874 SUMMARY OF CURRENT RESEARCHES RELATING TO

process. Where the object is entirely novel and the peculiar grainlike structure of the picture might cause erroneous conclusions, where there is a large amount of fine detail, or where, as in diatoms, the structure consists of a series of fine lines, or minute dots, elevations, or apertures, then this process is not so applicable. But even here it is serviceable if only the general appearance of the object is desired. One suggestion may be allowed to be offered—that in studying all process work the best effect is given if the picture is held at a rather greater distance from the eyes than the distance of normal vision, i. e. ten inches. By this means the attention of the observer will not be distracted by seeing the individual dots or points of which the image is made up, but he will still be enabled to appreciate all the delicate effects of light and shade.

There are several methods in common use for producing what are called half-tone prints. The bitumen process, though commercially but little used on account of its slowness, is one of the best for reproducing detail, especially since Valenta has discovered some valuable improvements, while the numerous so-called enamel processes which have recently been introduced, modifications of the gelatin process, are valuable in that they are rapid and cheap. The cheapness, of course, is one of the strongest recommendations for the use of any halftone process, as a cut which on wood might cost from 10 to 20 dollars could be put into a half-tone for 3 to 5 dollars. Another advantage is that the amount of detail in a picture would make no difference in the price of a zinc plate, it being sold for a certain price per square inch, while the cost of a woodcut would depend very largely on the fineness of the detail which was to be reproduced. This kind of illustration has already been largely used in medical work, and the A. M. M. J. a year or two ago republished some half-tone cuts by the author of photomicrographs of sections of woods, showing the capabilities of the process. For the representation of very delicate work, the numerous photo-gelatin processes are more satisfactory, as the minutest detail can be accurately shown, but they are much more expensive, and must be printed on special paper and presses. But graphic illustrations in some form add largely to the attractiveness of an article, as well as enabling the reader to comprehend at a glance the idea which is sought to be conveyed. Few can draw, but dry plates have made photomicrography almost a pastime, and the half-tone process offers, by all odds, the best mode of reproducing photographs with all the accuracy of the original, and with a minimum of expenditure of time and money, and it is to be hoped scientific periodicals will be led to make more use of this new method."

Drawing Photomicrographic Objects.\*—Dr. H. G. Piffard has done much to simplify the drawing of photomicrographic objects, by means of his application of the prism to the Microscope. His method is to insert a right angle prism by means of a short tube in the place of the eye-piece of the Microscope, and on one of the square faces of the prism another short tube to hold the ocular. The object then having been placed upon the stage and focused, a piece of plain drawing-

\* Anthony's Photographic Bulletin, xxiii. (1892) p. 516.
paper is placed under the ocular, and the room darkened, when a brilliant image will be apparent on the drawing paper. It is evident that in this way the artist has the advantage of perfect freedom, both of eyes and hands, and can trace the minutest detail with ease and accuracy.

The Microscope and a Hair.\*—Two different men were suspected of making an assault, but no proofs were forthcoming. A single hair which was found on the clothes of the victim finally became the clue to the mystery.

The hair was photomicrographed and compared with photomicrographs of the beard and hair of each suspect. There was entire lack of similarity and the case was about to be abandoned. The hair was pointed and had never been cut. Other facts pointed to its belonging to a smooth-haired and comparatively short-haired dog. Inquiry revealed the fact that one of the suspects owned such a dog. A fresh hair agreed in every respect with the specimen. The owner of the dog could not explain away the facts, and was convicted. He confessed indeed to having committed the assault.

### (5) Microscopical Optics and Manipulation.

Simple Method of Finding the Refractive Index of various Mounting Media.<sup>†</sup>—Mr. E. M. Nelson suggests the following method. "Provide two precisely similar equi-convex lenses, whose identical refractive index  $\mu$ , and radii r, are known, and cement them together with the mounting medium whose refractive index has to be determined. Now measure F, the principal focus of the combination, then the refractive index of the mounting medium.

$$\mu' = 2\,\mu - 1 - \frac{r}{2\,\mathrm{F}} \,\cdot$$

It is convenient to make the radii of the equi-convex lenses 2 in.: then

$$\mu' = 2\,\mu - 1 - \frac{1}{F} \,\cdot$$

Some examples might be of interest.

Let the refractive index  $\mu$  of the two equi-convex lenses be 3/2, and suppose that the combination has no focus, that is, that it behaves like a piece of plane glass, then

$$F = \infty$$
,  $\frac{1}{F} = 0$ , and  $\mu' = 2 \mu - 1 = 2 \cdot 0 \cdot$ 

If the principal focus of the combination  $\mathbf{F} = +2$ , then

$$\mu' = 2 \mu - \frac{11}{2} = \frac{3}{2},$$

or the same as that of the equi-convex lenses.

But if the principal focus of the combination F is negative, it must be measured in the same way as a concave spectacle lens, viz. by neutralizing it by a positive lens of equal focus.

- \* Microscope, xii. (1892) p. 176.
- † Journ. Quek. Micr. Club, v. (1892) pp. 8-9 (with an addition by Mr. Nelson).

If F is negative, the sign before the fraction will be changed. Example, let F = -2. Then

$$\mu' = 2\mu - 1 - \frac{1}{-2} = 2\mu - 1 + \frac{1}{2} = 2 \cdot 5.$$

The above method gives a greater range of readings for indices varying from 2.0 to 2.5, and consequently more accurate results than the simpler one of filling up a plano-concave lens with the medium, and covering it with a piece of plane glass. The formula for this latter plan being  $\mu' = \mu + \frac{r}{F}$ . The radius of the concave r might with

advantage be made 2 in., then  $\mu' = \mu + \frac{2}{F}$ .

If  $\mu = 3/2$ , and  $F = \infty$ ,  $\mu' = 3/2$ ; if F = 4,  $\mu' = 2 \cdot 0$ ; and if F = 2,  $\mu' = 2 \cdot 5$ ."

The following is a simple method of measuring the focus F := -On the stage of the Microscope place a slip with some scratch or mark on its *lower* surface. Screw a low power, such as a 1-in., on the nose-piece and bring this mark on the lower surface of the slip into sharp focus. Place the lenses with the enclosed medium on the substage of the Microscope, and by means of the substage rackwork carefully focus on the same scratch or mark on the lower surface of the slip the image of some distant tree or chimney-pot formed by these lenses. It will then be easy to measure the distance between the lower surface of the slip and the lenses, which will be the focus required. Note, if the Microscope is used in a vertical position it will be necessary to employ a mirror; care, therefore, must be taken to see that it is the *plane* mirror that is used; it would further be advisable to test the plane mirror by the sun's rays, as so-called plane mirrors are sometimes concaves of long foci, in which case they are unfit for use in the above measurements.

Abbe Measuring Apparatus for Physicists.\*—Dr. C. Pulfrich describes three measuring apparatus for the use of physicists, constructed by Prof. Abbe. The instruments are not new, but have not been fully described before. In their construction the following principles were mainly considered :—

(1) The measurement in all cases, both by contact as well as by sight-adjustment, is made by means of a scale, with which the distance to be measured is directly compared.

In this way all irregular and uncontrollable errors, such as are to be feared in the case of screws, are avoided.

(2) The apparatus is so arranged that the length to be measured forms a direct continuation of the scale which serves as the standard of measurement.

By this means the comparison of the measured length with the scale is made independent of the greater or less perfection of the mechanism by which the displacement is made.

In all three pieces of apparatus the divisions are engraved on plates of platinum or silver, which are only fixed at one end, so that they are free to expand in all directions. The standard scales are divided into

\* Zeitschr. f. Instrumentenk., xii. (1892) pp. 307-15. [The Society is indebted to Herr Zeiss for the use of the clichés of this article.]

1/5 mm., and every millimetre is marked by a figure. For readings below 1/5 mm. each instrument is provided with a micrometer-Microscope which is so arranged that two complete turns of the screw on the eye-piece correspond to one division of the scale, so that one division of the screw-head, which is divided into 100 equal parts, represents 1  $\mu$ . In the two instruments with contact adjustment the contact is effected by means of an agate pointer with spherical polished termination.

The contact micrometer represented in fig. 100 is intended to measure thicknesses up to 50 mm. by contact adjustment. The arm A, screwed to the base-plate of the apparatus, serves to support the Microscope, and also the arrangement for raising and lowering the scale. The platinum scale M, about 60 mm. long, is suspended at its upper end right and left between two points S. The lower end rests at the back against a projecting pin which is adjustable, so that the plane of the scale can be brought exactly into the line of displacement. This is the case when all the divisions of the scale during a complete displacement appear equally distinct, as seen in the Microscope. On the stand is a scale, divided in centimetres, on which the position of the zero point of the movable scale can be read with the naked eye. A smooth movement, as free as possible from friction, is obtained by means of two steel cylinders  $F_1$  and  $F_2$ , which pass through two three-sided openings.

The mechanism for raising and lowering the scale is easily understood from the figure. A cord fastened to the upper end of the cylinder  $F_1$  passes over the pulley R to the grooved wheel J, which is turned by the handles  $H_1$  and  $H_2$ . The weight of the cylinders and scale is partly counterpoised by the small weight G.

The object of which the thickness is to be determined is placed on a glass plate, 7 cm. in diameter and 1 cm. thick, let into the base-plate of the apparatus.

The second instrument, the Comparator, represented in fig. 101, serves for the measurement of divisions, gratings, spectra, star photographs, &c., up to 100 mm. It differs from the last in that the length to be measured is adjusted optically by a Microscope instead of by contact.

On a short strong tripod (fig. 101) is screwed a base-plate 16 cm. long, and about a hand broad. This carries the supports for the two Microscopes I. and II., of which I. is adjusted upon the scale, and II. upon the object. Scale and object are attached to a slide dovetailed into the base-plate, and displaceable from right to left.

Large displacements are made by hand by means of the knob on the right. The finer movement is effected by the screw  $S_1$ . The scale M is brought directly upon the slide A A. The object on the other hand lies on a second special slide B, which is connected with the main slide in such a way that it partakes of all the displacements of the latter, but can also be moved separately by means of the screw  $S_2$  on the left.

For the observation of objects with transmitted light, half of the main slide near the object slide is cut away along its whole length. The illumination in such cases is by means of a mirror beneath the baseplate. Further, in order to render possible the examination of square and circular plates, the support of Microscope II. is curved out so far that the foot is more than 50 mm. from the middle of the slide.

For many purposes it is useful to fit upon the slide B a rotating plate,

# 878 SUMMARY OF CURRENT RESEARCHES RELATING TO

on which the object is fastened. In the majority of cases, however, it is sufficient to fix the object with wax, and adjust by hand. The adjust-



ments of the comparator can be quickly made. First, the scale M must be moved by means of the two adjusting screws on its free end until no deviation with respect to the Microscope is noticed during a displacement of the slide through the length of the scale. Secondly, the double wire of the micrometer must be directed parallel to the marks of the division by turning the body-tube, after loosening the screws of the Microscopeholder. Thirdly, for avoidance of parallax, the image of the division must be brought exactly in the plane of the double wire.



For the adjustment of Microscope II. the slide is moved so far to the left that the first divisions of the scale come beneath the Microscope. The double wire is then directed parallel to the marks of the division, so that the double wires in both Microscopes are parallel. The course of the measurement is similar to that in the comparator.

The third instrument is the spherometer, shown in fig. 102. In principle it is the same as the ordinary spherometer, the radius of curvature R being given by the formula  $R = r^2/2 h + h/2$ , where h is the height, as measured by the instrument, of a dome with base of known radius r; but, as in Mr. E. M. Nelson's instrument, described in the October number



of this Journal, p. 670, for the usual tripod arrangement is substituted a ring on which the lens to be examined rests. The steel ring R fits into the plate P, supported on two strong uprights, and can be replaced by other rings of less or greater diameter. The base-plate of each ring is provided with a cylindrical boring which fits on to a cylinder screwed to the plate P, so that the central position of the contact point K is in all cases ensured. For greater security against wear and tear, each ring is formed of two edges about 1/2 mm. apart.

In other respects, viz. in the means for raising and lowering the scale and contact point, the apparatus is similar to the contact micrometer, and so far differs from Mr. Nelson's instrument, that no micrometer-screw, with its possible errors of back-lash, &c., is made use of. The error, common to all spherometers, and due to the fact that the contact circle of steel ring and spherical surface is not exactly coincident with the edge, so that the measurement of h for concave surfaces is too low, and that for convex surfaces too high, is found to diminish the greater the diameter of the ring. Accordingly, a ring of the largest possible diameter should always be used, and, in the case of lenses with surfaces of equal but opposite curvatures, the mean of the readings for the convex and concave surfaces should be taken as the value of h.

### (6) Miscellaneous.

The Microscope as an Aid to Physiology.—Canon Wilberforce having stated that the discovery of the circulation of the blood was due, not to Harvey, but "by means of putting the foot of a frog under the lens of a powerful Microscope," a writer in the 'Times'\* points out the indubitable claim of Harvey; at the same time he acknowledges that one link in the chain was wanting.

"Harvey knew that the blood found some channel by which it passed from the terminations of the arteries into the commencement of the veins, but he could not discover what that channel was. He conjectured that the blood percolated through the tissues, like water through earth; and at that time there was no Microscope in existence capable of showing either a capillary vessel or a stream of blood-corpuscles. Harvey's discovery was completed by Leeuwenhoek, in the second half of the century, by the aid of a Microscope which he made for himself in 1654, and which rendered visible, for the first time, the vessels and the bloodcorpuscles in the web of a frog's foot. Canon Wilberforce is therefore entirely wrong in his statement of what he calls a fact; and, if his fact were as he puts it, he would still be entirely wrong in his inference. If Harvey himself had seen a blood-stream in a capillary vessel, the sight would not have taught him the course of the circulation. The way in which blood passes from an artery to a vein through a capillary does not throw any light upon the nature and direction of its general circuits-first through the body, and secondly through the lungs, nor upon the function of the heart, by which it is propelled. The Microscope placed the keystone upon the arch of Harvey's discovery, but it could never have enabled him to construct the arch itself."

Twentieth Annual Report of Chief of the Division of Microscopy, U.S.A. $\dagger$ —Mr. T. Taylor reports :—" The work done during the past year relates in a great measure to the microscopical investigation of food adulterations, food-fats and oils, textile fibres, and edible and poisonous mushrooms. In relation to fibre investigation, I have had constructed, with your permission, a new machine of my invention for determining

\* Oct. 29th, 1892.

† Report of the Microscopist for 1891, pp. 405-6.

the general value and tensile strength of farmers' binder-twine, and for other purposes connected with farming interests. In these tests I have been courteously assisted by the officer in charge of the Bureau of Equipment of the Boston Navy Yard, and also by Mr. E. B. Balch, Superintendent of the National Cordage Company, New York City. This machine is now in good working order. A number of experiments have been made with it, and the results of the preliminary trials are herewith furnished. It may be well to state here that this machine has no relation to another machine invented by me and illustrated herein, designed solely for testing and comparing the relative strengths of fibres and of threads. There is also furnished in this report an interesting statement of preliminary tests, made with this machine, of four samples of foreign flax, showing their relative strength as compared with their relative cost per ton. These samples of flax were received from Mr. J. M. Anderson, Belfast, Ireland.

During the past year I have also devoted considerable time to investigating and reporting upon wool fibres, and have testified officially in the United States courts, for the Secretary of the Treasury, in cases where such examinations were pertinent to a question of dutiable merchandise. Valuable samples of foreign and native wools have been added to the collection in this division through the courtesy of Mr. E. A. Greene, Philadelphia, Pa.; also of Mr. John Consalus, Troy, N.Y., and others.

It may also be proper for me to mention that I have in progress the preparation of a large collection of models representing, by casts taken from nature, the edible and poisonous mushrooms of the United States, in groupings and otherwise, illustrating their manner of growth, development, colouring, and, as far as possible, their diversity of habitat. In this line of work enough has already been done to shape roughly an exhibit for the World's Columbian Exposition, which exhibit, it is desirable, should be as comprehensive and perfect as the one in the Museum at Nice, France, which shows the mushrooms prepared in plaster, life-size, and coloured after nature. In this way the public is enabled readily to compare one kind of mushroom with another, and to study them in all their stages of growth.

With the approval and co-operation of the Assistant-Secretary, I have, as already said, commenced my preparations for such an exhibit, which will be made as complete as the means placed at my disposal will permit."

## β. Technique.\*

Weichselbaum's Pathological Histology.†—Dr. A. Weichselbaum's work on pathological histology has special reference to methods of research, though there is a considerable amount of descriptive letterpress

\* This subdivision contains (1) Collecting Objects, including Culture Processes; (2) Preparing Objects; (3) Cutting, including Imbedding and Microtomes; (4) Staining and Injecting; (5) Mounting, including slides, preservative fluids, &c.; (6) Miscellaneous.

† Leipzig and Vienna, 1892. See Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) p. 255.

and of illustrations. For a first edition the illustrations are unusually numerous, for there are 221 of these, partly wood-engravings and partly zincographs, some being coloured. There are, as well, eight lithographic and photographic plates.

**Preservation of Teleostean Ova.**\*—Mr. Walter E. Collinge states that between October 1891 and July 1892, upwards of 80,000 ova have been examined at the St. Andrews Marine Zoological Laboratory, comprising some thirty known and four or five unknown species. He has made numerous experiments with various preservatives on a large number of these, and gives an account of the results obtained.

Killing.—The most satisfactory results were obtained by adding to a vessel containing the ova, with about an ounce of sea-water, three or four drops of a saturated solution of pieric acid, to which had been added 5 per cent. of hydrochloric acid. In this diluted solution they were allowed to remain for not longer than three minutes, during which time they were kept in motion by a pipette. When the ova remained for longer than the time stated, or when the solution was too strong, the yolk was generally ruptured, and considerable wrinkling took place in the zona radiata. In other cases the yolk became considerably contracted. Like results ensued if they were not well washed in fresh water before being transferred to the preservative fluid. After washing in dilute alcohol  $12\frac{1}{2}$ -25 per cent., a slight opacity followed. If killed in a saturated solution of 6 parts corrosive sublimate and 3 parts of glacial acetic acid they were also opaque when transferred to any of the following fluids.

Preservatives.—Some dozen or so of picric mixtures were tried, of which the following are the principal:—

(1) In equal parts of a saturated solution of piero-hydrochloric acid and 50 per cent. alcohol, ova of *Trigla gurnardus* shrank  $\cdot 1524$  mm.; the yolk was contracted and opaque; the oil-globule scarcely visible. In *Pleuronectes platessa* the shrinkage was slightly less,<sup>†</sup> being  $\cdot 1447$  mm.

(2) Saturated solution of picric acid, 1 part; glycerin, 1 part; 60 per cent. alcohol, 2 parts. *Motella mustella* shrank ·1524 mm.; the oil-globule was fairly distinct.

(3) Saturated solution of pieric acid, 2 parts; alcohol, 1 part. Results very similar to method 1. Shrinkage fully '1524 mm.; oilglobule poor and embryo indistinct.

(4) Saturated solution picric acid, 2 parts; 50 per cent. alcohol, 4 parts; 2 per cent. acetic acid, 1 part. Motella mustella and Trigla gurnardus; oil-globule and embryo indistinct; zona strongly wrinkled.

(5) Equal parts of saturated solution pieric acid, alcohol, and two per cent. acetic acid. The following ova were preserved in this fluid, of which the average shrinkage is given. The oil-globule, where present, was remarkably clear. Embryos very distinct. Ova previously prepared in other fluids, in which the oil-globule was scarcely or not at all visible, speedily came to view when allowed to remain in this fluid for five to twenty minutes.

\* Ann. and Mag. Nat. Hist., x. (1892) pp. 228-30.

† The average in all cases is given.

Species.			Average Shrinkage.			
///·//////////////////////////////////						mm.
Trigla gurnardus	• •	••	••	••	••	$\cdot 1447$
Gadus morrhua	••	••	••	••		$\cdot 1295$
<b>"</b> æglefinus	••			•••		$\cdot 1295$
,, minutus	••	••		••		·1143
Motella mustella	••	••	••			·990
Brosmius brosme		••	••	••		$\cdot 1371$
Hippoglossus liman	ndoic	les				$\cdot 1524$
Rhombus lævis	••	••	••			$\cdot 1371$
Arnoglossus laterno	ι		••	••		$\cdot 1447$
Pleuronectes plates	ssa					·914
Clupea spratītus			••	••		$\cdot 1143$

This was certainly the best of the picric solutions.

(6) Alcohol, 4 parts; 2 per cent. acetic acid, 4 parts; spirits of camphor, 1 part. The results here were very similar to the preceding fluid, but the embryos were not so distinct, owing to the slight opacity of the eggs; on the other hand, the shrinkage was very little. There are many objections to a picric solution which are here met. For general work, or for preserving large collections of ova, this is undoubtedly the best preservative I have used.

Species.					Aver	age Shrinkage.
						mm.
Trigla gurnardus	••	••	••		••	$\cdot 1371$
Gadus morrhua	••		••		••	$\cdot 1295$
,, æglefinus				••		$\cdot 1295$
,, minutus	••	••				$\cdot 1143$
Motella mustella		••				$\cdot 914$
Brosmius brosme						$\cdot 1143$
Hippoglossus limano	loid	les				$\cdot 1219$
Rhombus lævis	••			••		$\cdot 1143$
Arnoqlossus laterna						$\cdot 1219$
Pleuronectes platess	a					·914
Clupea sprattus						· 990

(7) Various mixtures of Kleinenberg's picro-sulphuric acid were tried, but the results in all cases were unsatisfactory.

(8) Very satisfactory results were obtained with 50 per cent. alcohol. The shrinkage was small, the oil-globule, however, was indistinct, and the dense opacity is a disadvantage.

(9) Perenyi's fluid stained the eggs a very dark violet. Diluted with 8 parts of 50 per cent. alcohol, very satisfactory results were obtained. The shrinkage averaged  $\cdot 1371$  mm., and the embryo in all the species experimented with showed well.

When ova were not permanently required they were allowed to remain in a 2 per cent. solution of acetic acid, or 4 parts of the same to 2 parts alcohol, and 1 part Perenyi's fluid; both mixtures gave good results. When the embryos were well advanced, they were allowed to remain in the former medium until considerable distention took place —about one hour or less. No effect was noticed upon the embryo until four or five hours.

It will be seen that the most satisfactory results were obtained by killing in picro-hydrochloric acid, and preserving in method 6.

Injection of a Mammal previous to Section-cutting.\* - Mr. H. Meller in giving a demonstration of his method, chose a rabbit and killed it by an injection of potassium cyanide into the mouth. "The apparatus and injecting mass being ready, immediately the animal was dead the thorax was opened and the apex of the heart cut off, so as to lay open the right and left ventricles; through the left ventricle a glass cannula was inserted into the aorta and fastened by a ligature tied round this vessel. To the glass cannula an ordinary indiarubber enema was attached, and by this means a continual stream of warm normal saline solution was driven through the vascular system, the blood and saline solution escaping by the right ventricle; as soon as this ran out clear, an ordinary glass syringe, charged with a gelatin mass coloured blue, was substituted for the enema. As soon as the injection began to pass out of the right ventricle a broad ligature was tied tight round the heart, just above the cut end, thereby preventing any more escape of fluid by the right ventricle. The cannula was still retained in the aorta, but the syringe being changed for another containing a gelatin mass coloured red, and not quite so fine as the preceding, this was injected into the arterial system so as to drive the first injection as completely as possible into the veins. During the injection everything was kept under warm salt solution, and when the operation was completed the animal was laid aside for one or two hours to allow the mass to solidify. The demonstrator then explained that at the completion of this time the parts could be prepared for section-cutting, or the animal preserved in 90 per cent. spirit or other preservative. The following are the formulæ for the various solutions used during the demonstration :---Normal saline solution: salt 7.5 grm., water 1000 ccm. Gelatin mass: soak gelatin in water till soft, then melt over water-bath. Red injection: gelatin mass mixed with carmine dissolved in ammonia. Blue injection: gelatin mass mixed with freshly precipitated Prussian blue."

#### (1) Collecting Objects, including Culture Processes.

Bacteria-fishing Apparatus.<sup>†</sup>—Dr. Schrank fishes out a specimen from a particular colony for inoculation purposes by means of a needle fitted to a metal case, like an objective, in which the needle is substituted for the lens. With a low power the particular colony is focused at the intersection of cross-threads, and then the lens replaced by the needle, which is lowered down until it reaches the colony. The colony is again observed in order to make sure if the needle has touched it.

Influence of Filtration on Liquids containing Microbic Products.<sup>+</sup> M. Arloing has made some experiments to ascertain what effect earthenware filters have on the composition of fluids containing microbic secretions. The liquid used was the juice of beetroot, after it had been fermented in silos. This fluid was passed through new Chamberland filters, and it was found that a considerable percentage of proteid

\* Journ. Brit. Dental Assoc., xiii. (1892) pp. 581-2.

† Zeitschr. d. Allgem. Oesterr. Apothekervereines, 1892, No. 14. See Centralbl.
f. Bakteriol. u. Parasitenk., xii. (1892) p. 312.
‡ Comptes Rendus, cxiv. (1892) pp. 1455-7.

and hydrocarbonaceous matter was retained. This retaining power became diminished by use, and also after sterilization of the bougies, and in all cases the toxicity of the filtrate was lowered. Hence the author considers that mineral filters have a distinct hygienic value, but are not sufficiently accurate for scientific purposes.

Permeability of the Chamberland Filter to Bacteria.<sup>\*</sup>—Dr. E. von Freudenreich finds that the Pasteur-Chamberland filtering apparatus will produce germ-free water for at least eight successive days, and that therefore it may be used for household and laboratory purposes provided that it is sterilized every week, and that the temperature of the water does not exceed certain limits  $(15^{\circ}-18^{\circ})$ .

Procedure for Obtaining Germ-free Water.<sup>†</sup>—Drs. V. and A. Babes describe an apparatus for obtaining germ-free water; the vessel itself is



of zinc or glass, having the shape of an Erlenmeyer's flask, and capable of holding 20-40 litres (fig. 103). At the bottom is a pipe with stop-cock for letting off the water, and at the side an aperture, closed by a screw-tap, for cleansing purposes. The vessel having been filled with water, 3-6grms. of powdered alum are put in, and then stirred up with a flat perforated piece of wood, or by means of a perforated mixer turned by a handle. When thoroughly stirred up the mixer is removed and the vessel covered with a cap. In 18-20 hours the water is drawn off by the tap at the bottom. It is advisable to let the first halflitre 1un off. The principle on which the apparatus and procedure are founded is that of sedimentation and decantation, and though alum acts very well other substances may be used, such as sulphate of iron or chalk. A similar result was obtained by currents of air, but the details are not given. The authors think that the results of their methods

are very encouraging and infinitely superior to any of the filtration methods, all of which are condemned as being worse than useless. The

- \* Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) pp. 240-7 (1 fig.).
- † Tom. cit., pp. 132-8 (1 fig ).

main objection to filters is, that after having been used for a few days the filtered water contains more germs than the unfiltered. Of course all the results were tested bacteriologically.

Trambusti's Culture Apparatus.-We give a figure of this apparatus (fig. 104), a description of which appeared in the last number of the Journal (p. 691).

Keeping the Inoculation Wire.\*-Dr. H. C. Plaut suggests that medical men desirous of obtaining cultivations of disease germs can keep the inoculating platinum wire and glass rod, previously sterilized of course, within the test-tube containing the cultivation medium, the test-tube being plugged with cotton-wool and covered with a caoutchouc cap.

Method for Cultivating Anaerobic Bacteria.<sup>†</sup> — Herr Hesse cultivates anaerobic bacteria in test-tubes on solid media in the following way:-In a test-tube already filled with a solid medium, cotton-wool is loosely

pushed in for a distance of some cm., the open end of the tube is then immersed under mercury, and hydrogen introduced into the tube. The tube is then withdrawn, the cotton-wool removed, the medium inoculated, and then the open end again immersed under the mercury, and hydrogen again introduced. For liquid media, or those which may become so, and for plate cultivations, a bell-jar is used.

Apparatus for Cultivating Anaerobic Bacteria.<sup>‡</sup>-Dr. A. H. C. van Senus uses a very simple and convenient apparatus for cultivating anaerobic bacteria. In a glass tube, the diameter of which is about 6 mm., a U-shaped bend is made, and one end drawn out to a point. The narrow end having been covered, and the wide end plugged with cotton, the apparatus is sterilized. To fill the tube the narrow end is inserted in gelatin or agar, previously inoculated, and the medium sucked in through the broad end. When sufficient has reached the U-shaped bend the narrow end is sealed up. In order to obtain a colony for inoculation purposes the tube is carefully cleaned with H<sub>2</sub>SO<sub>4</sub> and sterilized water, and then having been notched with a sterilized file, the piece on which the colony desired is situated, is removed. The disadvantage of the method is that it does not afford any chance for microscopical observations, although a hand lens is available. But by a modification the device may be adapted to plate cultivations, by simply blowing a bulb in a tube with diameter of about

\* Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) p. 203. † Zeitschr. f. Hygiene, xi. No. 2. See Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) p. 173

<sup>†</sup> Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) pp. 144-5.



6 mm. The bulb should then be flattened out so that its thin sides are not more than 2-3 mm. apart. This apparatus is sterilized and used in much the same way as the former, and its only disadvantage is that it must be broken in order to isolate a colony.

Capsule for Cultivating Anaerobes.\*—Dr. L. Kamen describes a neat little contrivance for cultivating micro-organisms, which he has used very successfully in experiments with tetanus. It consists of a flat circular glass capsule, the side of which forms a broadish edge, on which the cover, of equal diameter, rests. In the edge are cut out, on opposite sides, two narrow grooves with a slope towards the bottom of the capsule. In the cover are two small holes.

The apparatus is easily manipulated. After having been filled with some cultivation medium in the usual manner, the margin of the cover and the flat edge of the capsule are smeared with vaselin, and the two adjusted so that the holes in the cover are over the oblique grooves. The apparatus is then filled with gas (H;  $CO_2$ ) which replaces the air by driving it out at the opposite opening. When filled the cover is just slipped aside so that a hermetically closed cavity is immediately made.

Three illustrations are given, one showing the upper surface, and the two others sections of different portions.

Cultivating Gonococcus.<sup>†</sup>—According to Herr E. Wertheim, Gonococcus can be easily cultivated if human blood serum be used. The serum is solidified by adding sterilized gelose to it. From these cultivations typical gonorrhœa was excited in the human urethra (five cases). The gonococcus retains its virulence for some weeks provided it be protected against desiccation. It thrives better in absence than in presence of oxygen.

Inoculation experiments on animals showed that it was capable of exciting peritonitis, although different kinds of animals evinced unequal degrees of susceptibility.

Sig. A. Risso<sup>‡</sup> succeeded in cultivating gonococci, from a recent case of gonorrhœa, on placenta blood serum, both with and without the addition of agar or gelatin. Inoculations with pure cultivations in the anterior chamber of the eye of a rabbit gave positive results.

Pure Cultivations of Tubercle Bacilli from the Human Corpse.§ —Dr. Wünschheim states that he succeeded in obtaining pure cultivations of tubercle bacilli by using some pia mater from a case of acute tubercular meningitis. The cultivation medium was blood-serum, and three out of five were successful.

Isolating a Rennet Ferment from Bacteria-cultures. ||--Prof. H. W. Conn has succeeded in separating the rennet ferment from the proteolytic

\* Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) pp. 296-8 (3 figs.).

† Prager Med. Wochenschrift, 1891, Nos. 23–4. See Annales de Micrographie, iv. (1892) pp. 359-60.

<sup>†</sup> La Riforma Medica, 1892, No. 118. See Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) p. 205.

§ Prager Med. Wochenschr., 1892, No. 25. See Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) p. 205.

|| Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) pp. 223-7.

enzyme allied to trypsin, both of which are found in milk after the action of certain kinds of bacteria, in general those having a liquefying action on gelatin. Sterilized milk is inoculated with the bacterium, which is allowed to grow for 7-10 days after coagulation has occurred. The milk is then carefully shaken up with distilled water, and next passed through a porcelain filter. The clean filtrate contains all the soluble ferments. It is frequently colourless, but is often of an amber hue, or even brownish. From this material the soluble ferments may be precipitated by alcohol, collected and dried, the dried precipitate having the property of coagulating milk and peptonizing gelatin. The rennet is separated from the pepsin by acidulating the filtrate with 0.1 per cent. of sulphuric acid, and then adding common salt in excess. When the fluid becomes saturated with salt a snowy scum forms on the surface. This is nearly pure rennet ferment. The scum is then removed and dried. The snow-white powder is rennet mixed with salt, and possibly some other impurities.

The rennet ferment seems to be more quickly developed at moderately low  $(20^\circ)$  temperatures than at higher ones  $(35^\circ)$ , which are more favourable to the pepsin ferment.

#### (2) Preparing Objects.

Investigation of Origin of Vascular Germs in the Chick.\*-M. L. Vialleton opened the eggs in water to which a small quantity of salt solution had been added, and which had been heated to 35°; the blastoderm was rapidly cut out and placed on a glass plate; it was then treated with a 1 per cent. solution of silver nitrate, washed with distilled water, and put (in darkness) in 70 per cent. alcohol for from six to twelve hours. On being removed from the alcohol it was put in an alcoholic solution of borax-carmine, in which it remained till it was sufficiently stained; it was then washed with 70 per cent. alcohol slightly acidulated with hydrochloric acid, dehydrated completely in 90 per cent. and absolute alcohol, and mounted in dammar. Owing to the reduction of the nitrate of silver, the boundaries of the epithelial cells were well marked, while the borax-carmine stained the nuclei in such a way that a fine preparation was obtained, in which a number of interesting histological details could be made out. The only fault of this method of preparation is that the reduction of the nitrate of silver sometimes goes too far, and sometimes the ectoderm becomes folded.

Spermatogenesis of Gryllotalpa.<sup>†</sup>—Dr. O. vom Rath obtained best results with Flemming's chrom-osmo-acetic acid, "Hermann's fluid," and a mixture of picr-osmo-acetic acids. As stains, the most successful were alum-cochenil (24 hours in warm temperature), and safranin-gentianorange.

Examination of Gills of Palæmonetes varians.<sup>‡</sup>—Mr. E. J. Allen found that the gills of this Crustacean were somewhat difficult to preserve; the use of sublimate and alcohol failed to give satisfactory results. With strong Flemming's solution he was able to obtain preparations

\* Anat. Anzeig., vii (1892) pp. 624 and 5.

1892.

<sup>†</sup> Arch. f. Mikr. Anat., xl. (1892) pp. 102-32 (1 pl.).

<sup>‡</sup> Quart. Journ. Micr. Sci., xxxiv. (1892) pp. 75 and 6.

# 890 SUMMARY OF CURRENT RESEARCHES RELATING TO

which showed both cell-outlines and protoplasmic structure in an excellent state of preservation. The objects must remain in the solution for from three to fourteen hours, according to the degree of softness of the chitin. After hardening in alcohol, the specimens were stained with Delafield's hæmatoxylin, and were removed from Flemming's solution to water, and after a few minutes transferred to crude pyroligneous acid, where they remained for nearly twenty-four hours; by this last process (von Mährenthal's method), the osmic acid is reduced in the tissue, and no further staining is required. After dehydration, the objects were sunk in chloroform, and then placed for several hours on the water-bath in a mixture of chloroform and paraffin, after which they were imbedded in paraffin, and cut in the usual way.

Examination of Nervous System of Ascaris megalocephala.\*— Herr R. Hesse made transverse and longitudinal sections of this Nematode. The material, which was fixed by sublimate solution, water at 60°, chrom-osmic-acetic acid, 1/2 per cent. osmic acid, picrosulphuric acid, and 96 per cent. alcohol, was found to be useless, as the nerves crumpled up greatly. By chance in one sublimate and one water preparation a part of the nerve was not crumpled. In the rest, the ganglion-cells were alone made clear by this method. Others which were placed fresh, for a day, in 1/2 or 1 per cent. solutions of chromic acid, and for a week in chromates, gave poor results when imbedded in paraffin, but better when placed in celloidin. The best results were got with specimens that had hardened for a long time in alcohol. Grenacher's borax-carmine was used for staining.

Preparation of Embryos of Strongylus paradoxus.<sup>†</sup>—Herr B. Wandollech observed the development of living ova in weak salt solutions at a temperature of  $30^\circ$ ; to prevent evaporation the cover-glass was surrounded on three sides by wax, while the fourth remained open to allow of the addition of fluid. To make preparations of the complete egg, a specimen of the worm was placed without any fluid on a very thin slide, and cut through in the middle; the uterus escaped with the fluid of the cœlom. The slide was next flooded with the fixing fluid heated up to 70°. The albumen coagulated, and while the embryos were preserved they were at the same time firmly fixed to the slide, and could be treated like sections attached by albumen. Borax-carmine was used for staining, and it was found necessary to leave the eggs in it for some In order that the cell-boundaries might be made distinct, Herr time. Wandollech made use of a method suggested to him by Prof. F. E. Schulze. On removing the preparations from absolute alcohol, he placed them in picric acid dissolved in xylol; the objection to this method is that it requires much practice and patience.

Examination of Strongylus convolutus.<sup>‡</sup>—Dr. H. Stadelmann was, owing to the transparency of this worm, able to make *in toto* preparations of it; these were put in glycerin, and not in Canada balsam, as in the latter reagent specimens become in time quite opaque; this was not owing to insufficient removal of the water, as animals which had been

<sup>\*</sup> Zeitschr. f. Wiss. Zool., xlv. (1892) pp. 549 and 50.

<sup>†</sup> Arch. f. Naturg., lviii. (1892) pp. 127-9. ‡ Tom. cit., pp. 152 and 3.

for months in absolute alcohol, renewed almost daily, exhibited the same phenomenon. Cold bichloride of mercury was used as a fixing reagent; Lang's solution generally destroyed the cuticle. Other fixing materials are not suitable for Nematodes, as they affect the subsequent staining.

To avoid crumpling, the hardening was effected in Schulze's dialyser, after which the pieces were left for some time in absolute alcohol. They were then treated, not with xylol, which causes crumpling, but with chloroform. This was evaporated in an oven, and paraffin was continually added; when this had set, sections of 3  $\mu$  thickness were cut. Sections were stained with borax-carmine or hæmatoxylin; gold-chloride was used to make the cell-boundaries more distinct. In order to follow out the finest details of the nervous system chrom-osmic acetic acid was used, followed by acetic, the pieces being about six hours in the former, and twenty-four in the latter fluid.

Investigation of Ctenophora.\*-Dr. P. Samassa succeeded, notwithstanding the statements of Chun and Hertwig, in making satisfactory sections of Ctenophores. He effected this by the celloidin-paraffin method which he modified as follows; the object was removed from absolute into a mixture of equal parts of ether and absolute; ordinary celloidin was cut up into small pieces, and dried in the oven so as to completely remove all the water. Every day a piece was added to the alcohol and ether mixture in which the object was, so that after ten days the solution was glairy. As the long period of remaining in the solution is not in the least harmful to the object, a slow increase in the amount of celloidin is much to be recommended, as the danger of curling up is thus avoided as far as possible.

Preparation of Budding Hydroid Polyps.<sup>†</sup> — The specimens studied by Herr A. Lang, which were sent him from Naples, were preserved in 70 per cent. sublimate alcohol, those from Genoa were partly in absolute, and were partly killed by hot sublimate and preserved in 90 per cent. alcohol. The sections were stained with picrocarmine (after Ranvier), alum-cochineal and hæmatoxylin; double staining was effected by picro-carmine (in toto) and subsequently by hæmatoxylin and Lyon's blue. Alum-cochineal stained the nuclei well. The Hydræ were preserved with hot watery or alcoholic sublimate solution, and partly with Rath's mixture of picric, osmic, and acetic acids.

Von Koch's Petrifying Method.<sup>‡</sup>—Dr. C. Röse discusses the method of petrifaction which von Koch used in studying silicious sponges, corals, and the like, and points out that L. A. Weil, in using it for the study of teeth, omitted to "steam" very slowly, and thus obtained artificial results. For "Weil's sheath" is certainly an artificial product, and can be produced by a misuse of Koch's ingenious method.

Observation and Vivisection of Infusorians in Gelatin.§-Herr P. Jensen finds, as Prof. Stahl suggested, that a gelatin solution is most useful for studying infusorians and the like. Their movements

- \* Arch. f. Mikr. Anat., xl. (1892) p. 158.
- Żeitschr. f. Wiss. Zool., xlv. (1892) pp. 366 and 7.
  Anat. Anzeig., vii. (1892) pp. 512-9,
  Biol. Centralbl., xii. (1892) pp. 556-60.

3 0 2

### 892 SUMMARY OF CURRENT RESEARCHES RELATING TO

are inhibited while their life is preserved. Three grams of white gelatin are dissolved by heating in 100 ccm. of water; the result is a stiff jelly at the temperature of the room. In this, about 3 per cent., gelatin solution, which may be diluted if desired, the movements of *Paramæcium* and *Urostyla* are prevented, but the cilia and the contractile vacuoles remain active for hours. For observing the movements a solution of 1.5 per cent. is most useful; for vivisection experiments  $\cdot 8-1$  per cent.

### (3) Cutting, including Imbedding and Microtomes.

Hard Section Cutting and Mounting.\* — Mr. J. W. Dunkerley recommends the following process, which is specially adapted for dealing with large specimens, such as horse's teeth, as well as smaller ones. "Sections are cut off the tooth by means of a thin copper disc, fitted in the ordinary manner on to a dental lathe, and revolving in a tin trough which contains water and fine corundum powder. This thin disc is now replaced by a thick one, with the same trough and contents ; the sides of this disc are used as a lapidary stone to grind thinner these sections, one side of which is next polished on a soft stone (Water of Ayr) under running water, this surface being afterwards secured to a glass slip by thick Canada balsam. The grinding of the section on the thick copper disc is now proceeded with until the section is thin enough to see the structure; then proceed to polish this surface on the Water of Ayr stone until all details are seen under the Microscope, when after careful washing the section is mounted."



Rapid Method of Dehydrating Tissues before Infiltrating with Paraffin.<sup>†</sup>-Mr. G. L. Cheatle describes a modification of Soxhlet's

- \* Journ. Brit. Dental Assoc., xiii. (1892) p. 581.
- † Journ. Pathol. and Bacteriol., i. (1892) pp. 253-5 (1 fig.).

extraction apparatus which he has devised for dehydrating pieces of tissue (fig. 105). It consists of a flask capable of holding 25–30 oz. of fluid; from the neck of the flask proceeds a tube through which spirit vapour at first, and afterwards liquid alcohol passes to a vessel in which the piece of tissue to be dehydrated is placed. From near the bottom of the flask proceeds another tube to the dehydrating vessel, and the latter is joined to the former by a siphon. Hence, if the flask be filled with alcohol above the level of the side tube and heated in the usual way, the pure spirit will pass along the upper tube to the siphon-vessel containing the tissue to be dehydrated, and then from this vessel back through the siphon to the flask. Hence this continuous distillation means rapid dehydration. The water is absorbed by putting a handful of quicklime in the flask with the spirit.

The only other detail which may be mentioned is that from the middle of the lower or side tube which connects the flask with the siphon-vessel is a tube coming off at right angles. This seems to act chiefly as a safety valve. All the parts are connected with rubber tubing, and fixed in the usual way.

Taken altogether, the apparatus seems to be very ingenious, and worth a trial, as its action is continuous and automatic, and the waste quite a minimum.

Imbedding Vegetable Objects in Celloidin.\*—According to Herr W. Busse it takes 3-4 weeks to properly prepare an object by the celloidin process. The object should be perfectly dehydrated and free from air, and be gradually impregnated with celloidin by successive immersion in solutions of increasing thickness. At least three different solutions are required. The next step is to imbed the object in thick solution in paper capsules. These are then placed in alcohol for twenty-four hours, by which time the celloidin mass is ready for sectioning. The author deals with sectioning and the after treatment of the sections, and shows how to remove the colloidin by a modification of Chauveaud's procedure.

Paraffin Infiltration by Exhaustion.<sup>†</sup>—Mr. A. Pringle advocates the infiltration of tissues with paraffin by means of exhaustion on account of celerity; certain and complete infiltration; certain removal of the solvent; absence of distortion of the tissue elements; avoidance of overheating, and economy.

The object may be fixed by immersing in saturated solution of  $\text{HgCl}_2$  for about 12 hours, and then washing in running water for a like time. After this it is passed through 30, 50, and 70 per cent. alcohols successively for 24 hours apiece. The pieces are preserved till wanted in 70 per cent. alcohol. Or the objects may be fixed and hardened in Müller's fluid followed by the alcohols as above. When required the pieces are transferred from 70 per cent. alcohol to pure methylated spirit, and absolute alcohol (twice) each for 24 hours. Chloroform is then put under the spirit by means of a pipette or syringe, and left for 24 hours. The mixture is replaced by

\* Zeitschr. f. Wiss. Mikr., viii. (1892) pp. 462-75. See Bot. Centralbl., li. (1892) p. 292.

† Journ. Pathol. and Bacteriol., i. (1892) pp. 117-9.

pure methylated chloroform, and the containing vessel left loosely stoppered on the paraffin stove till all traces of alcohol have vaporized. Then the tissue is placed in melted paraffin, and as soon as it is warmed through, it is placed under an air-pump. The plate of the air-pump is smeared with glycerin or lard; over this is laid a sheet of indiarubber, and this also treated with glycerin or lard.

As soon as the air is exhausted from the receiver bubbles begin to rise, and as long as they do the pumping may be continued, though it is well, after a little pumping, to let the air into the receiver at least once. This is done by having a tap between the air inlet and the plate. The paraffin must be kept melted the whole time. If the paraffin solvent has been chloroform, the whole process takes about fifteen minutes, but if the preparation have been cleared with benzole and cedar oil, the time required is a little longer.

The pieces of tissue dealt with are supposed to measure  $1 \times 1\frac{1}{2}$  in. by 1/4 in. thick. For further details and other hints the original must be consulted.

Beck's Double Slide Microtome.—This microtome introduces a new feature in section-cutting which Messrs. Beck consider of some importance. The single slide microtome of the usual type, when used with the long diagonal knife, possesses this disadvantage, that the razor being supported at one end only is liable to have considerable spring or give at its further end, thus decreasing its stability, and rendering it more difficult to cut the finest sections.

The double slide microtome has a strong frame to carry the knife, which runs on two parallel circular bars of steel. The knife is clamped upon both sides of this frame, the object to be cut being between. The



knife, which has a blade 7 in. long, is thus supported at both ends, and the whole extent of the blade can be used. The position of the razor can be varied at will; the clamps can be placed at any position on the frame, so that the knife can be placed at right angles to the direction of cut, or in any diagonal position.

The clamps are on a new principle. The blade of the knife rests upon a ledge on the lower surface of the clamp, and is held in position by three screws at the top, two at A (fig. 107), in front of the ledge, and one at B, behind the ledge; thus by altering the relative position of these screws, the angle of the blade may be varied; the under portion of the knife, which is flat, may be placed so as to be quite horizontal, or may have a slight inclination. In addition to this, these clamps are capable of carrying almost any razor.

The object-holder is carried in a ball clamped in a long plate, which

plate is pivoted at one end of the microtome and raised by a micrometer screw at the other (fig. 108).



The ball when unclamped gives a motion for moving an object in all directions till it is in the required position. The milled head which raises the object is divided into 100 divisions, and has an index, each

## SUMMARY OF CURRENT RESEARCHES RELATING TO

division of the scale raising the object 1/5000 in. ( $\cdot 0002$ ). It is obvious that the section is slightly wedge-shaped, but the amount is so extremely trifling, that expert section-cutters have informed the makers that they consider it immaterial.



Mayer's Section-stretcher.—The section-stretcher shown in fig. 109 can in this new form be used on each side of the knife, and is easily fitted on the back. The long rod e is adjusted, partly by hand and partly by means of the screws f, so that it is parallel to the knife-blade

and projects half beyond it; it is then lowered by the screw g until almost in contact with the blade.

Thanhoffer Knife.—Fig. 110 represents a knife for Microscope sections, which is provided with a water-spray.

**Preserving Fluids.\***—Dr. F. Krasser recommends, as a highly antiseptic preserving fluid for vegetable preparations, a mixture of 1 vol. acetic acid, 3 vols. glycerin, and 10 vols. of an about 50 per cent. solution of sodium chloride. It has also the property, in many cases, of preserving the natural colour of the section. The author also refers to the property of a 1 per cent. alcoholic solution of salicyl-aldehyde of fixing the colour of pigments, as, e. g. that of the chromatophores of *Solanum Lycopersicum*.

### (4) Staining and Injecting.

Methods of Staining Medullated Nerve-fibres.—The following is an account of the remarks made by Dr. Beevor at the meeting in October. Dr. C. E. Beevor, after stating that the title of his paper was not quite correct, as not only the fibres but the cells also were stained, briefly described the methods of work with the following stains :—

Weigert's Acid Fuchsin Method.—The material is hardened in a 3 per cent. solution of potassium bichromate for six weeks, then imbedded in celloidin, cut, and the sections placed in a saturated aqueous solution of acid fuchsin. They are then washed in water, and transferred to absolute alcohol with 1 per cent. of caustic potash until the grey matter is of a lighter colour than the white. The sections are again washed in water before mounting. This method may also be used for staining *in toto*, and afterwards imbedding in paraffin.

Weigert's Hæmatoxylin Method (1884).—The material is hardened for from two to six months in potassium bichromate, imbedded in celloidin, sectioned, and transferred direct for 24 hours into a solution of hæmatoxylin (hæmatoxylin 1 grm., absolute alcohol 10 ccm., water 90 ccm.). The sections are then washed quickly in water, and passed into the following solution:—Ferricyanide of potash  $2\frac{1}{2}$  parts, borax 2 parts, water 100 parts, in which they can be left until no more colour comes out. Afterwards wash in water, dehydrate in absolute alcohol, clear in oil of cloves, and mount in Canada balsam.

The medullated fibres are stained dark blue, and the grey matter and fibrous tissue a pale yellow.

Staining *in toto* with this method is not a success as the hæmatoxylin will not penetrate.

Prof. Weigert recommends the addition of a minute quantity of an alkali such as lithia carbonate to the hæmatoxylin solution, which turns it a dark purple colour.

Weigert's Copper Method (1885).—Weigert puts the imbedded block into a solution of acetate of copper half saturated, and then cuts, stains, and treats with the ferricyanide solution.

Dr. Beevor said that he usually used this method after sectioning, placing the sections in a solution of acetate of copper, washing off the excess in methylated alcohol, then staining and developing.

\* SB. K. K. Zool.-Bot. Gesell. Wien, xlii. (1892) p. 56.

By this method the fibres are much blacker and the substance a dark orange.

Pals Modification of Weigert's Method was brought out about three or four years ago. The hardening process in this method is extended to a longer period, sometimes up to six months. After staining the sections in the hæmatoxylin solution, they are rapidly washed in water, and transferred to a 1/4 per cent. solution of permanganate of potash till there is a differentiation, then into a watery solution of potassium sulphite 1 per cent., oxalic acid 1 per cent.; this bleaches all but the medullated fibres, which appear quite black; carmine can then be used.

Success with this method is harder to obtain than with Weigert's procedure. It does not show the different forms of fibres so well, but is useful on account of the double stain.

Schafer's Improvement of Pal's Method.—The material is hardened from four to six weeks, and, after cutting, the sections are put into Marchi's fluid (1 part of a 1 per cent. solution of osmic acid, and 2 parts of a 3 per cent. solution of potassium bichromate). Then wash quickly in water and stain with the hæmatoxylin solution (hæmatoxylin 1 grm., acetic acid 2 ccm., water 100 ccm.). Develope afterwards as by Pal's method.

This is a very good method for quickening the hardening process, and for sections which have been too long in alcohol after potassium bichromate.

Marchi's Method for Degenerate Medullated Fibres.—Harden several pieces in a 3 per cent. solution of potassium bichromate for a week, then cut a thin slice and put in Marchi's fluid for a week. Imbed in celloidin, section, and develope.

By this method only the degenerate fibres are stained black.

Lewis's Method for Staining Cortical Cells by Anilin Blue-black.— Sections are made from a fresh brain, frozen, stained by .25 aqueous solution for one hour, afterwards washed in water, and developed.

Golgi's Method.—The material is hardened in potassium bichromate, put into a solution of silver nitrate, and then washed in water: The sections are dehydrated in alcohol, and mounted in Canada balsam.

Imbedding for Examining Tissues for Tubercle Bacilli.\*—Sig. G. Cirincione dehydrates the material to be sectioned in absolute alcohol and then transfers it for twelve hours to bergamot oil. It is then soaked in melted cacao butter for 24 hours and kept at a temperature of 35° C. Thus impregnated the mass is set by cooling under a stream of water. It is then quite ready for cutting, which in summer should be done immediately. The sections are next transferred to bergamot oil which dissolves out the cacao butter, and then to absolute alcohol, after which they may be stained in the usual manner. The advantages of this method are that micro-organisms contained in the tissues are not exposed to physical or chemical damage during manipulation, that it is expeditious, and that it stains "plasma-cells" quite well.

Method for Making Paraffin Sections from Preparations stained with Ehrlich's Methylen-blue.<sup>†</sup>-Mr. G. H. Parker, at the suggestion

of Prof. F. E. Schulze, has devised a method for making these sections. The elements of the nervous system of a Cray-fish were stained by injecting 1/10 to 1/20 ccm. of a 0.2 per cent. aqueous solution of methylen-blue into the ventral blood-sinus; in about fifteen hours many cells and fibres were stained. Preparations thus made retain their colour for only about an hour, unless they are treated with reagents which precipitate the methylen-blue; the best to use is corrosive sublimate used as a cold, concentrated, aqueous solution. The tissue must now be dehydrated in a solution composed of 1 grm. of corrosive sublimate and 5 ccm. of methylal. It is next put in a mixture composed of two parts xylol, one part pure methylal, and one part of the dehydrating mixture of sublimate and methylal. After a short time in this it is placed in a considerable quantity of xylol for four or five days. After this it may be either mounted in xylol-balsam and studied as a transparent object, or imbedded in paraffin and cut in the usual manner. The sections should be fixed to the slide with Schällibaum's collodion, and not with Mayer's albumen which discharges the colour. Preparations or sections made in this way are serviceable for several weeks.

Staining Sympathetic Nerve-cells.\*-Prof. A. Van Gehuchten recommends the following method; the ganglia are to be extracted from animals killed with chloroform and put at once into a mixture of 5 parts of 1 per cent. osmic acid and 20 parts of 3 per cent. bichromate of potash. Leave in the dark for three days; then wash rapidly with distilled water and place in a 0.73 per cent. solution of nitrate of silver. If there is not a slight precipitate add to the silver bath a few drops of the osmio-bichromic solution. Leave the piece in the silver bath in the dark for at least two days. Again wash rapidly with distilled water, and again immerse in the osmio-bichromic solution. After three days and another rapid washing replace in the silver bath for at least two days. Then imbed in celloidin.

Technique for Botanical Investigations.<sup>†</sup>—Herr J. af Klercker describes a method of preparing vegetable microtome sections without previous fixing or saturating with paraffin. The object is placed at once in solidifying paraffin, and cut with the knife placed very obliquely and moistened with water. For dry herbarium material, articles of commerce, &c., he recommends that, before placing in the paraffin, they should first be immersed in cold or boiling water, ammonia, or dilute potash-ley; very brittle objects may be saturated with glycerin-gelatin. For making permanent preparations of objects containing tannin, the author recommends the following fixing solutions:--(1) Flemming's chrom-osmic acid; (2) a mixture of 1 part Kleinenberg's picrinsulphuric acid, and 1 part 5 per cent. solution of potassium bichromate; (3) a mixture of 1 part picrin-sulphuric acid and 1 part concentrated solution of cupric sulphate; the second for not more than one day; the third from one to two days. In thick sections the tannin-cells appear brown-red by the first and second, green by the third method.

Staining Cell-nucleus of Pollen-grains.<sup>†</sup>—Herr A. Meyer uses for this purpose a substance which he calls chloral-carmine, consisting of

‡ Ber. Deutsch. Bot. Gesell., x. (1892) p. 363.

<sup>\*</sup> La Cellule, viii. (1892) p. 87.
† Verhandl. Biol. Ver. Stockholm, iv. See Bot. Centralbl., lii. (1892) p. 56.

0.5 grm. carmine, 20 ccm. absolute alcohol, and 30 drops of hydrochloric acid, which is heated for thirty minutes in a water-bath, when 25 grm. chloral hydrate are added. This solution, filtered after cooling, stains the nuclei of pollen-grains in ten minutes an intense red. It can be used also for the nuclei of pollen-tubes grown on gelatin, since it liquefies that substance.

Sterilization of Drugs for Hypodermic Use.\*—Sig. D. Marinucci has found that many hypodermic solutions, such as freshly prepared 1/2-1 per cent. strychnine sulphate, curara, eserin, atropin sulphate, hydrochlorate of morphine, and 100 per cent. quinine chlorate were examined by bacteriological methods, and found to contain a greater or less (but always considerable) number of living germs which apparently are not all of a harmless nature.

Solutions sterilized by means of heat and non-sterilized solutions were injected into rabbits and white rats, and in some cases frogs and mice.

The experiments showed that the therapeutic value of strychnine, curara, quinine, and eserin were unaffected after sterilization by heat. The action of morphine and atropine was diminished by heat sterilizing and, therefore, the dose should be increased if the solution have been sterilized. Eserin was completely altered. The solutions of eserin and atropin were best sterilized by preparing them with a 1:1000 sublimate solution, by which their therapeutic properties were unaffected. It would be better however to renew the solutions every fourteen days, although they will keep for a long time. The author was unable to hit upon any practical method for sterilizing morphine without damaging its therapeutic action.

New Method for Staining Microscopical Preparations.<sup>+</sup>—Dr. W. Swiatecki describes a device for staining microscopical preparations, which is said to be both practical and satisfactory. It merely consists in covering the preparation with filter-paper soaked in the staining solution. The procedure is suitable to dried layers of fluid and to sections. It is carried out on a slide in preference to cover-glasses. The filter-paper should be not quite as big as the slide, and to this when applied in one or more-layers the staining fluid is dropped on. When it has acted for a sufficient length of time it is washed off, and the layer or section treated in the usual manner, either for decoloration, for counter-staining, or for dehydration.

It is almost unnecessary to remark that when a layer of fluid, e.g. sputum, is made on a slide, the superficial extent of the layer needs several cover-glasses.

Simple Method for Staining Tubercle Bacilli in Sputum.<sup>‡</sup>—Dr. P. Kaufmann uses boiling water as the decolorizing agent instead of acid, and his method is as follows:—

The sputum is dried on the cover-glass and then fixed in alcohol or over the flame, after which it is stained in the usual manner with phenolfuchsin. The cover-glass is next waved about in boiling water for  $1\frac{1}{2}$  to

<sup>\*</sup> La Riforma Med., 1891, p. 805. See Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) pp. 282-3.

<sup>†</sup> Centralbl. f. Bakteriol. u. Parasitenk., xii. (1892) pp. 247-9.

<sup>‡</sup> Tom. cit., pp. 142-3.

3 minutes. The preparation may then be contrast stained or examined at once in water, the tubercle bacillus appearing dark-red on a whitishgrey background.

In order that the staining should succeed well, the layer of sputum should be as thin and even as possible.

FABRE-DOMERGUE—Note à propos de la méthode bactériologique au bleu de prusse de M. Solles. (Note on M. Solles' Method of staining Bacteria with Prussian Blue.)
Compt. Rend. Soc. Biol., 1892, p. 407.

MOORE, V. A.—Observation on Staining the Flagella on Mobile Bacteria. Bacteriol. World, Battle Creek, Mich., 1891-92, pp. 115-9.

- SOLLES-Méthode nouvelle de recherche bactériologique; ses premières applications. (New Method of Bacteriological Research; its first applications.) Journ. Méd. Bordeaux, 1891-92, pp. 258-9.
- SQUIRE, P. W.—Methods and Formulæ used in the Preparation of Animal and Vegetable Tissues for Microscopical Examination, including the Staining of Bacteria. London, 1892, 8vo, 100 pp.
- STRAUS, J.—Sur un procédé de coloration à l'état vivant des cils ou flagella de certaines bactéries mobiles. (On a Process for Staining in the Living State the Cilia or Flagella of certain Mobile Bacteria.)

Compt. Rend. Soc. Biol., 1892, pp. 542-3.

WURTZ-Technique Bactériologique. (Bacteriological Technique.) Paris, 1892, 8vo.

#### (5) Mounting, including Slides, Preservative Fluids, &c.

Use of a Substitute for Canada Balsam.\*—Dr. A. M. Edwards, who has long sought for a substitute for Canada balsam for mounting objects for microscopic use, and has, in fact, employed a hundred different media, rejected them one by one until he got the one which he now describes.

"I use the gum thus, or frankincense, which is the gum or balsam of the Pinus tæda L. (loblolly, or old field pine), which is found in Virginia and southward, common. In Florida it is very common, constituting the 'Pine Barrens' of that State. It was described in the 'Dispensatory of the United States of America,' sixteenth edition, 1889, by Wood and Bache, and by Wood, Remington, and Sadtler as from the Pinus Australis Mich. (P. palustris Mill.), and P. tæda Linn. It is dissolved in alcohol. A saturated solution is made by adding ordinary alcohol to a large quantity of the gum and set by for a day or so until it is dissolved. The clear solution, which is darker than balsam, is poured off, and three parts acid to one of oil of cinnamon is added to nine [parts of the solution]. This is the solution that is used for mounting. The gum thus is more highly refractive than Canada balsam alone, and when we add to it oil of cinnamon we use liquid of the highest refractive powers that we can use. To use it, we dry the substance, diatoms, or other substance in the cover or slide, and add with a dipper (an iron wire is good) a drop or two of the solution. We then warm it until the alcohol is flown off and bubbles formed are driven off, and the cover is pressed on the glass slide and the whole cooled. The slide is then cleaned with solution of ammonia (I use a weak household ammonia) or carbonate of soda, or borax and water. A ring of asphaltum or gold size can then

\* Science Gossip, 1892, p. 236.

### 902 SUMMARY OF CURRENT RESEARCHES RELATING TO

be turned around the cover, and the mounting is done. It will be found that the mounting is easy as compared with Canada balsam, for no turpentine is used, and as no sticky residuum is used the cleaning is also easy. I think that those who use it will be pleased with the results, and Canada balsam mounts be sent to the limbo."

The Rev. Father Thompson's High Refractive Medium.\*—Mr. E. M. Nelson, who some years back exhibited a beautiful slide of diatoms, mounted in a very dense medium by the Rev. Father Thompson, has now, through the kindness of that gentleman, been able to communicate the recipe of the composition. He still has the same slide in his possession, and, so far as it is possible to judge, it has remained unaltered. He therefore begs to commend Father Thompson's high refractive medium to the especial notice of the Quekett Club as the best thing that has been done in that direction.

"Take flower of sulphur, bromine, and arsenious acid in the proportions of 8, 10, and 12 respectively by weight. Dissolve the sulphur in the bromine with gentle heat in a thinnish test-tube about 6 in. long. Over a small Bunsen jet add small portions of the arsenious acid, boil, and let the condensed vapours of the mixture cool and fall down the sides again. Be very careful that these do not escape. If none of these have escaped, the proportions given will be correct; but if they do escape, probably a spot more bromine will have to be added to keep the mixture clear.

No mechanical directions can be given beyond these. Success is very much like that of a cook in his preparations, and the eye and understanding must regulate the proceedings. When made, the mixture should be about the consistence of toffee, and much the same in appearance. It should be handled with a piece of platinum wire. The more arsenic the better, and a grain or two of the metal itself may be coaxed in towards the end so long as the mixture remains clear. If properly made this will last, so far as I know, for ever."

Substitute for Glass for Covers and Slides for the Microscope.t-Dr. A. M. Edwards writes :-- "I think the price of slides and covers for microscopic use is enormously high, and as they can be made of a substance much cheaper, and at the same time possessing properties which glass has not, viz. being unbreakable, that it should be known. In using celluloid, which is wood rendered soluble in ether and alcohol with gum camphor, for films for microphotography, I was struck with some of its properties, that made me think it could be used in microscopy. It is transparent, almost as transparent as glass, unbreakable, the weight is very little, making it especially valuable when sending by post, and therefore occupying very little room, which can thus be dispensed with. It is strong as wood, and stronger, has no fibre, and can be cut readily with scissors. I really wonder that it has not been used before for slides and covers. It can be obtained with a ground surface as well as plain, and the cost, which is a great item, is next to nothing. Very thin celluloid films are commonly used for instantaneous coverers, and this can be employed for covers, whilst the thicker kind used for

\* Journ. Quekett Micr. Club, v. (1892) p. 123.

† Science Gossip, 1892, pp. 235-6.

ordinary photography makes capital slides. In fact, I have some an inch square, which I use in this way, mounting it temporarily in a glass slide for use on the Microscope. Let all microscopists try it, and they will not repent."

#### (6) Miscellaneous.

Microchemical Reactions of Cork and Cuticle.\*—Herr A. Zimmermann discusses the mode of detection of these substances by the use of osmic acid, alkannin, and cyanin. Osmic acid, in a 1 or 2 per cent. solution, with warmth, causes a rapid and intense brown or black colour of all suberized membranes. When tannins are present, it is recommended to destroy them by eau-de-Javelle. Lignified are stained much more slowly than suberized membranes by osmic acid. A solution of alkannin in 50 per cent. alcohol, when warm, causes an intense colour in all suberized membranes. It is better to treat first with eau-de-Javelle. A very good reagent is obtained by mixing equal volumes of glycerin and a concentrated solution of cyanin in 50 per cent. alcohol; after previous treatment with eau-de-Javelle it brings out an intense blue staining of lignified and suberized membranes.

Microscopical Examination of Coal.<sup>†</sup>—In his researches Herr J. Wiesner uses a mixture of a concentrated aqueous solution of potassium bichromate and an excess of chromic acid, adding then sufficient water to dissolve the separated sulphuric acid. Oxidizable substances are by this process coloured a yellowish red which finally passes into green. Amorphous carbon will resist this reagent for months; its opaque particles form the chief ingredient of soot, of coal, of anthracite, and of black charcoal. Brown coal and brown charcoal are intermediate stages between pure carbon and cellulose, and a similar substance is found in anthracite. Soot contains, in addition, resinous substances which are rapidly dissolved by chromosulphuric acid. Graphite consists of a readily oxidizable substance and of small black granules which resist the reagent for two months. The black particles found in human lungs are identical with soot.

Microscopical Examination of Textile Fabrics.<sup>†</sup>—Herr J. Vinzenz publishes a handbook for the microscopical examination of the fabrics of commerce, both animal and vegetable. The microscopic characteristics of the different fibres are described and delineated, and the microchemical reactions are given.

\* Zeitschr. f. Wiss. Mikr., ix. (1892) pp. 58-69. See Bot. Centralbl., lii. (1892) p. 84.

+ SB. K. Akad. Wiss. Wien, ci. (1892) pp. 379-418.

t 'Anleit. z. Mikrosk. Unters. d. Gespinnstfasern,' Cottbus, 1890. See Bot. Centralbl., lii. (1892) p. 153.