MICROSCOPY.

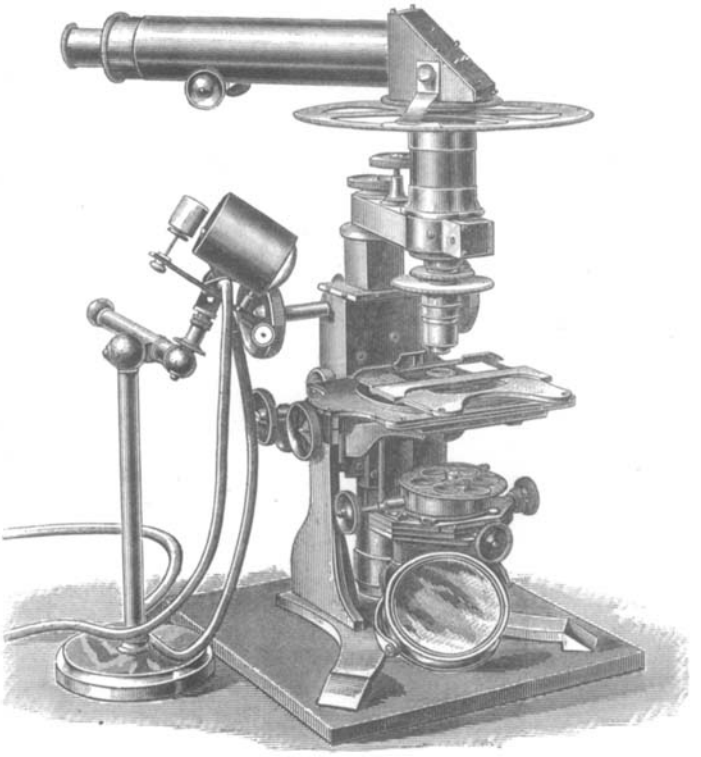
a. Instruments, Accessories, &c.*

(1) Stands.

Braham's Universal Microscope.—The following description has been communicated to us by Mr. Philip Braham, of Bath:—

"The original design of the instrument was based on the most improved Microscope, devised by the late Andrew Ross: but the modi-

FIG. 50.



fications I have made with a view to facilitating special investigations are considerable.

Fig. 50 shows my application of a rectangular prism, giving the

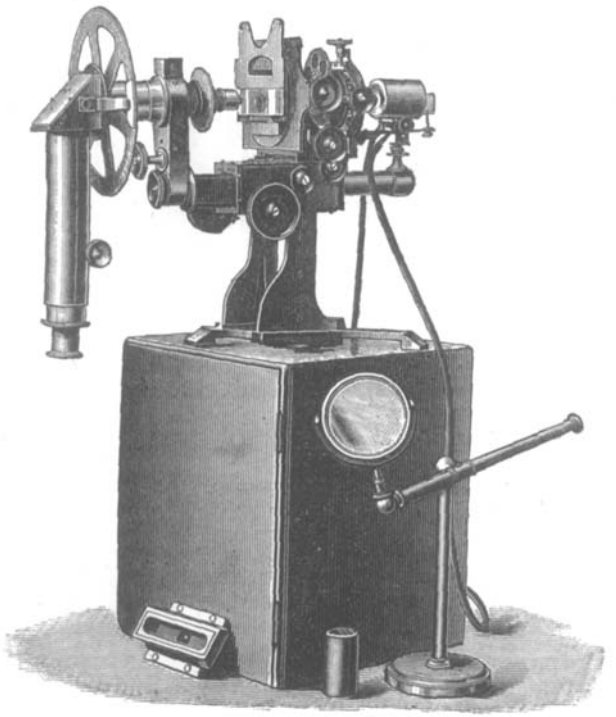
* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous.

observer an easy position for examining objects in liquids, and the means of measuring the angles of crystals.

The angles of crystals are measured by cross-wires in the focus of the eye-lens, a divided circle attached to the body of the instrument, and a Vernier attached to the side of the brasswork, carrying the rectangular prism, which is adjusted by three screws, so that the hypothenuse is exactly at an angle of 45° with the optic axis. The adjustment is made by placing on the stage a slip of glass, ruled with fine cross-lines, which are made to coincide with the cross-wires in the eye-piece.

The divided circle can be turned by rotating the tube at right angles

FIG. 51.



to the optic axis. The magnified image of the crystal also rotates, the angles being measured by the coincidence of the sides of the crystal with the cross-wires in the eye-piece.

This arrangement is also useful in observing phases in polarization, the tube carrying the polarizing prism on the substage being rotated by clockwork, and four pins making electrical contact and ringing a bell,

by which every quarter revolution is marked, and attention called to the changes visible.

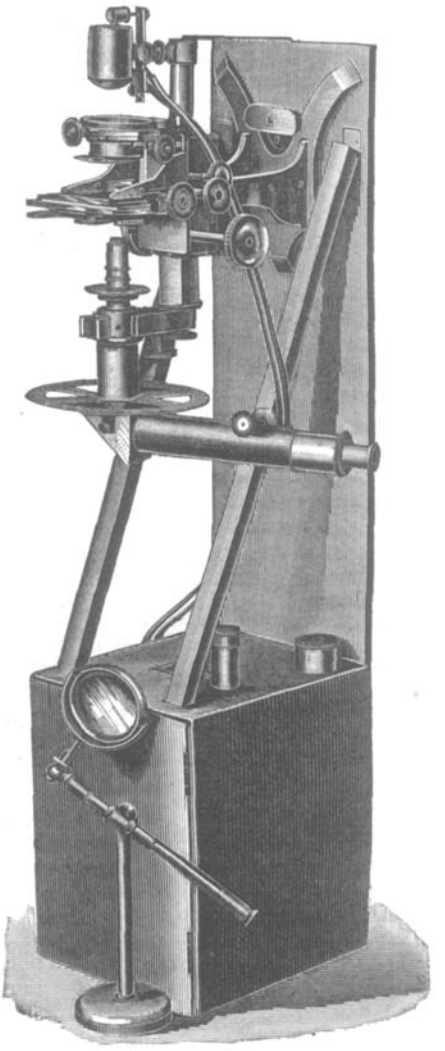
The limelight illuminator is shown in position for illuminating opaque objects, and a light from the mirror through coloured glass gives a good background for a variety of objects.

The limelight apparatus shown is conveniently clean and devoid of smell, and gives out very little heat. It can be used for oblique, opaque or transparent illumination, and can be varied in intensity. It consists of a diminutive limelight on a condenser stand, with an adjustable plano-convex lens in front. By varying the distance of the plano-convex lens in front of the limelight either convergent, divergent, or parallel rays can be obtained and projected in any direction.

Fig. 51 shows the instrument in position to project an image of an object on a sheet of paper on the table for sketching; the limelight being attached in the place of the mirror.

Fig. 52 shows the adjustment of the instrument in an inverted position. A board is attached to the box, and two struts are applied; the Microscope is then clamped to the upper part of the board, the feet fitting into corresponding notches in the board. This enables the observer to examine objects from beneath, whilst objects in liquids and tubes are seen free from cylindrical aberration by immersing the tubes in a cell shown on the table in Fig. 51.

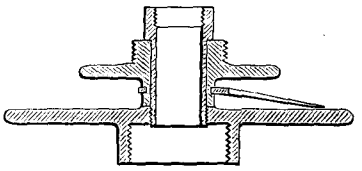
FIG. 52.

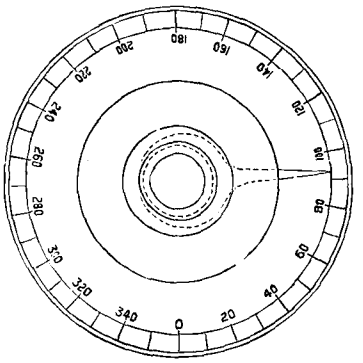


The interior of crystals or gems can be microscopically explored by immersing them in equally refractive liquids.

Fig. 53 shows my rotating nosepiece, consisting of a screw fitting to the objective end, and a divided circle fitting to the objectives and rotating. An index pointer sprung on to the nozzle shows the angle of rotation; the other end of the rotating tube is adapted to receive the analysing prism or a double-image prism, which can be used for measuring the angles of crystals by rotating the magnified extraordinary image round the ordinary. It can also be used in testing objectives."

FIG. 53.





Dumaige's "New Model of Microscope." *—M. Fabre Domergue describes and figures what he calls "un nouveau modèle de Microscope," constructed by M. Dumaige of Paris. We cannot, however, discover anything new in the instrument, and the only points to remark on are:—(1) the teeth of the rack are of ovoid section, and are set obliquely (a plan we believe not previously adopted in a French Microscope); (2) the carrier for the Abbe condenser is of reduced size, so as not to require the stage to be raised too much above the base; and (3) the pivot on which the condenser carrier turns is fixed to a slight prolongation from the right posterior corner of the stage, which the author considers to be "a very great advantage; it constitutes for the hand that works the micrometer-screw a kind of natural support, and allows the fingers much ease and suppleness in using the screw."

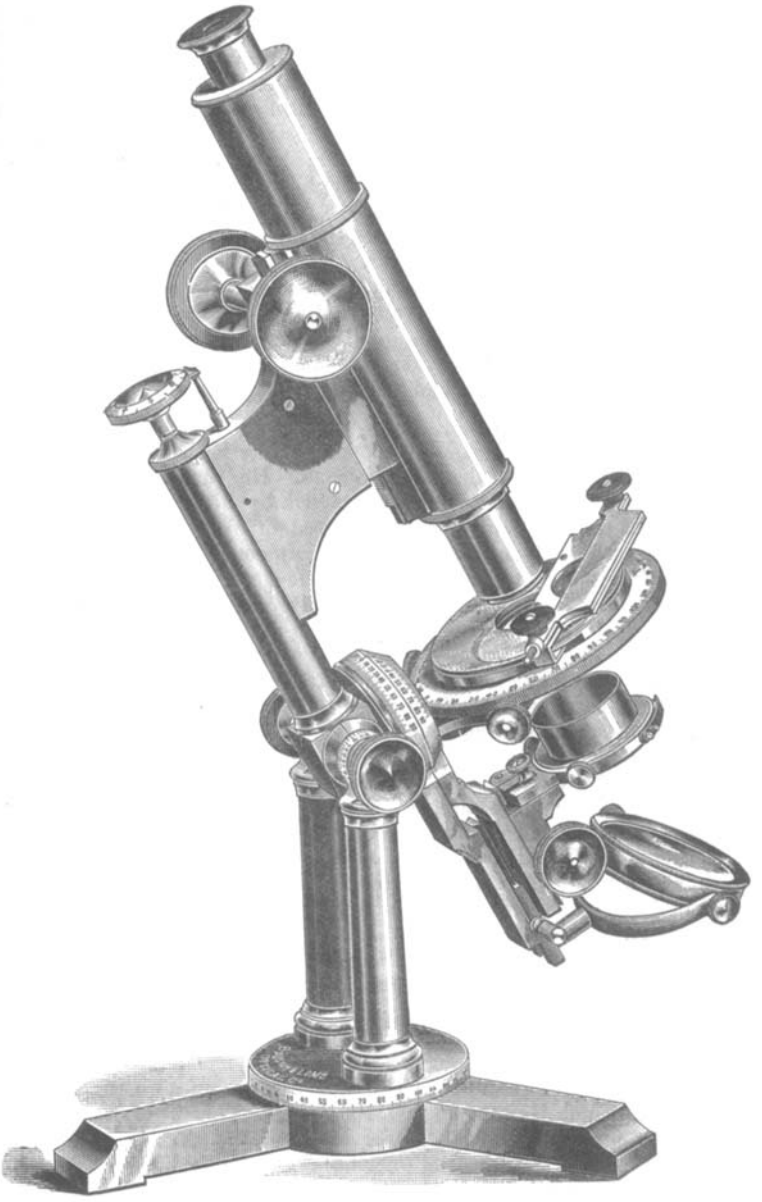
M. Fabre Domergue refers to condensers as having been "completely neglected ten years ago!" The introduction of the Abbe condenser, he says, imposes upon constructors the necessity of modifying the old models of stands so as to allow of the introduction of condensers beneath the stage.

Hart's Microtome-Microscope.†—The following is the abstract of a paper by Dr. C. P. Hart, as printed in the Proceedings of the American Association for the Advancement of Science, under the title of "*A new, cheap, useful, and quickly constructed adjustable Microtome.*"

"This instrument is nothing more nor less than a Bausch and Lomb "Microscope-stand, converted into a microtome by the following changes: "—Having removed the substage, the slide-carrier clips, the objective "adapter, and the draw-tubes, a suitable razor-blade is permanently fixed "to the slide-carrier, so as to have a corresponding universal lever move-

* Ann. de Micrographie, ii. (1890) pp. 164–7 (1 fig.).
† Proc. Amer. Assoc. Adv. Sci. for 1885 (1886) p. 356.

FIG. 54.



HART'S MICROTOME-MICROSCOPE.

"ment parallel to the glass stage. The imbedded substance is then carried "down the main tube of the instrument (which is placed in a horizontal "position) until it presses gently against the microtome-knife, when it is "fixed in position within the tube by means of the main draw-tube, the "diaphragm of which, either directly or by means of a small wooden "cylinder, is brought in contact with the distal extremity of the substance "to be divided, and this acts as a plug or follower to retain it in position "within the tube. Then, having moistened the knife, and, if necessary, "the substance to be operated on also, the slide-carrier, and with it the "microtome-knife, is made to pass through a sort of revolving cutting "motion, by which the sections are made. These sections may be made "of any degree of delicacy by means of the micrometer-screw attached to "the instrument."

The notion of converting such a Microscope into such a microtome seemed to be so unique in the novelty of its originality (it is difficult to hit on the exactly appropriate designation) that we imported the instrument from the United States, and give an illustration of it in fig. 54, which shows the razor-blade and slide-carrier in the form designed by Dr. Hart.

Alterations in Nobert's Microscope.*—Herr Kayser describes some alterations made on a Nobert Microscope. He particularly mentions the simple reading arrangement constructed by him, which is just as serviceable as a microscopical one composed of eye-piece and objective, but it does not invert the image. This arrangement consists of a small tube, containing only a thread and a plano-convex lens. Close to the eye comes the thread stretched horizontally, and then the lens, with convex side in front, at such a distance that the image of the thread is distinctly seen by the passage of the rays through the lens, and reflection at its plane silvered surface. A narrow strip of the silvering is removed in a direction passing through the centre, and at right angles to the thread. Consequently, when the distance of the tube is suitably adjusted, the eye can see a division through this central space. In order to have the division, but not the thread, more strongly magnified than in this, the simplest case, a second plano-convex lens of suitable focal length can be added immediately on the plane silvered face of the lens. Here two equal lenses of 10 mm. diameter and 25 mm. focal length are combined. On the thread end of the tube a white paper screen inclined at 45° with central aperture is fitted for the illumination of the thread. This small reading arrangement is fastened to the object stage, while an ivory rod with a range of 80 mm. divided into half millimetres, and fixed vertically on the Microscope-tube, can be displaced with the tube. A screw with large drum divided into 50 divisions serves to raise or lower the stage by slow degrees. Since the tenth of the division can be easily read, an arrangement is thus attained which, over a very large interval (80 mm.), gives an adjustment and a measurement which is exact up to 1/1000 mm. This is of importance, for instance, for microscopical measurements of the refractive indices of transparent plates. By means of the fine screw, the error of the divisions on the scale can be tested, and it is especially serviceable in

* Schrift der Naturforsch. Gesells. Danzig, vii. (1890) pp. xi.–xii.

adjusting objectives of short focal length and immersion systems, which must otherwise be done by testing, and consequently with danger to the apparatus. In determining the refractive index of a transparent plane parallel plate, Herr Kayser proceeds as follows. The refractive index is $= \frac{D}{D - d}$ where D and d are given by three readings on the scale, when the adjustment of the Microscope is made:—1. On the support of the plate. 2. On the upper face of the plate, after it has been put on the stage. 3. On the support as seen through the plate. The readings 1 and 2 give D, the readings 1 and 3 d. This method is, however, not sufficiently precise. Another and more exact method, with experimental proof, will be given later.

(3) Illuminating and other Apparatus.

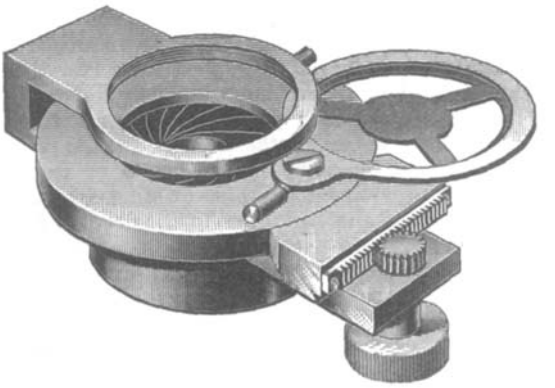
Mayall's "Jewelled" Fine-adjustment.—At the April meeting of the Society, Mr. J. Mayall, junr., referred to an improved form of fine-adjustment constructed and exhibited by Messrs. Powell and Lealand, for the production of which he was himself chiefly responsible. He said that during the past ten or twelve years, several forms of fine-adjustment had been brought to the notice of the Society, but the principal aim in most of them had been economy of production or lowness of price, without regard to improving on the best existing forms. In the new form exhibited the chief aim had been to construct a fine-adjustment that should combine extreme sensitiveness of action with accuracy and probable durability, beyond what had previously been attained. With this view he had carefully considered every known form of fine-adjustment, and had selected that of Messrs. Powell and Lealand, as representing the highest type of construction yet devised, with which to test the possibility of improvement. The essential feature in the improvement was the application of what watchmakers would term a "jewelled movement." The whole of the contact surfaces by which the fine-adjustment was actuated consisted of polished steel and agate, the intention being to reduce the friction as much as was consistent with steadiness of motion. The perfection and durability of jewelled mechanism was a great feature in the highest class of clocks and watches; the most delicate parts of Nobert's ruling machine were jewelled, as were also the bearings in Dr. Hugo Schröder's feeling level for testing the accuracy of plane surfaces. Those who were familiar with Powell and Lealand's fine-adjustment, as previously constructed, would understand the extreme difficulty of improving the mechanism substantially, for it was the outgrowth of long experience and of the most conscientious devotion of expert mechanicians to the task of providing a perfect focusing movement. No other fine-adjustment had reached the same high standard of construction, which was probably due to the fact that during the fifty years that had elapsed since its first production, the makers had kept steadily to the same system, only varying the minor details of the mechanism as experience critically suggested in the direction of improvement.

The application of polished steel and agate bearings throughout the mechanism was intended to reduce the friction, and thus render the

action more sensitive without introducing unsteadiness. The result attained was undoubtedly an improvement on the whole system, though the cost would probably limit the application to the few instruments required for very special and difficult investigations in microscopy. For high-class photomicrographic work, or where preparations had to be retained under observation for long periods of time, the new mechanism should be particularly useful, for the greater solidity of the general construction clearly pointed to greater precision of action and increased stability.

Messrs. Bausch & Lomb's Condenser Mounting.*—We give a figure of a condenser mounting with iris diaphragm recently designed by Messrs. Bausch and Lomb. This mounting provides a movement for the

FIG. 55.



diaphragm by rack and pinion. It has in addition a recess for receiving central stops and blue glass.

It can be attached to an adjustable substage or to a substage fixed to the stage, and may be used with the high and low angled Abbe condenser.

New Stage Micrometers.—At the May meeting of the Society, Mr. E. M. Nelson called attention to a new stage micrometer, produced by Messrs. Powell and Lealand, and so excellently ruled as to be worthy of remark:—"It comprises 100ths and 1000ths of an inch, and 10ths and 100ths of a mm., there being 10 divisions of each set; the finer divisions of ·001 in. and ·01 mm. being placed in the centre, the ·01 in. being on the one side, and the ·1 mm. on the other, respectively, a guiding line being ruled at right angles to them. The lines are fine, 1/30,000 in., and are blackened in, and mounted in balsam. The lines are straight, and evenly ruled. With regard to the spacing, I have made exhaustive comparisons with fine micrometers by Rogers and Zeiss, and some others not quite so perfect. Upwards of 240 screw micrometer

* Amer. Mon. Micr. Journ., xi. (1890) pp. 25–6.

measurements were made, and the work carried on under hypercritical conditions. An account of these may be of interest. First, a magnification of 1200 diameters by means of a suitable immersion lens was employed for the finer ruling, and for the coarse a dry 1/6 × 600 diameters; the screw micrometer was on an independent mounting. Care being taken with regard to the illumination, &c., a critical image of the lines was obtained. The order in which the lines were taken was from left to right, as seen in the instrument; each interval was then designated by consecutive letters of the alphabet. The intervals were then most carefully wired, and each value set down under its corresponding letter; when the ten spaces were finished they were meaned.

It was then easy to see which interval differed from the mean, and to calculate how much. In the same way comparison can be made with any other scale, it matters not whether it is ruled in inches or mm. It is most important that both the instrument and the observer be tested. To this end I proceeded as follows. The screw value of 20 intervals on a badly ruled scale was written down as above, the paper was then put away, and the operation performed again.

On comparing the two papers, the screw values of seven intervals were identical, 12 different by one division, and one by two divisions. This error of two divisions occurred in the interval H, the first reading being 1033, and the second 1031. On careful re-examination of this interval, I came to the conclusion that the first reading was the bad one, and that the true value was 1031 or 1032. On substituting this last value in both sets of readings, the 20 intervals meaned precisely alike, viz. 1038. As this forms a suitable illustration of the work, I append the two columns. With the exception, therefore, of the interval H, the screw readings may be taken as true to ± 1. The point, therefore, we have to determine, is the value of ± 1. The mean 1038 being the value in divisions of the screw-head, for 50 μ, the value of one division consequently = ·000001897 in., or less than 1/500,000 in.

This might be called 'the constant of the instrument, and observer.' We next have to find the greatest errors of the intervals from the mean; G is the greatest, and S the least. Calculation shows that G is 1/20,000 in. too large, and S 1/40,000 in. too small.

But, on returning to Powell's scale, we find a much closer agreement than this. Taking the ·001 in. first, we find the mean to be 628·0. Three out of the ten intervals agree to that mean to ± 1: this being 'the constant of the instrument and observer,' they are without sensible error. Four intervals agree to ± 2, which is less than 1/300,000 in.; two lines B and H agree to ± 3, which is less than 1/200,000, and one interval G is + 4, viz. 1/157,000 in., too large. Now, as we found that ± 1 was the limit of observation, we may say that the scale, with the exception of B, G, and H, has no sensible error. Practically speaking, G is the only interval that is out, and its error is small in comparison with other scales.

The next scale is the 1/100 mm.

The ·01 mm. is too small a quantity to treat in the above way; it must be left until we have objectives as perfect as those we have at present, but of double their power.

All that can be done is to take several of the divisions. Eight sets

of three each were measured on Powell's new scale: the variation from the mean was less than 1/200,000 in. Rogers' is a very well ruled scale; it is, however, difficult to observe, the lines being without pigment, and it is mounted dry. The lines under these circumstances present the usual black and white diffraction images. It is, on that account, very difficult to maintain an equable focus during measurement. In Rogers' scale, the greatest error is in interval G, where it amounts to four divisions, or somewhat less than 1/100,000 in. Thirteen out of twenty intervals have practically an insensible error. One cannot speak with the same certainty with regard to this plate as to the others, because of the focal difficulty. Different readings gave discordant results; therefore, in this case, more must be allowed for the 'constant of the observer and instrument.' With regard to the 1/10ths of a mm. on Powell's scale, they were examined by a power of 600 diameters by a dry lens. The mean was 987; six intervals had no sensible error, but C and G had an error of three divisions, which is equivalent to 1/100,000 in.

Rogers gave a very similar result.

The error of the interval D, in the Zeiss scale, was 1/30,000 in.

I next compared the length of the mm. on the three scales, that is Powell's, Rogers', and Zeiss', with each other. I detected a slight but insensible difference of $\pm$ 1. All that now remains to be done, is to compare the inch and the mm. scale on Powell's plate. By measurement, we found that 30 μ gave a screw value of 741·25; therefore, the value for ·1 mm. would be 2470·8, and the value for 1/1000 in.

$$\frac{\cdot 001 \times 2470\cdot 8}{\cdot 003937} = 627\cdot 59.$$

The value actually measured was, as we saw above, 628·0; here again there is no sensible discrepancy. In conclusion, I feel sure that such an accurately ruled micrometer, and one so clear to read, will prove extremely useful to microscopists at large.

Before closing, I would like to bring to your notice a screw micrometer made for me by Mr. Powell, which contains some slight modifications from the usual forms, which practical experience has suggested to me.

First, with regard to the lens portion, I have substituted a compensating positive for the old form of Huyghenian or Ramsden. This yields far better images when making measurements with apochromatic and ordinary objectives. I have so arranged it that the compensating eye-lenses of different foci are interchangeable. In fact, no special lens is required, you use your ordinary working eye-piece, whatever that one may be. This is, of course, a great advantage: bacteria, for instance, require a high-power eye-piece micrometer, while such a power would be useless on an ordinary object.

Therefore, the ability to regulate your eye-piece power to the object to be measured, will meet a long felt want.

Next let me say that I entirely disapprove of having two movable threads; at the outset 'the constant of the instrument' would be doubled; moreover, I am confident that a movable zero is a mistake.

I have, therefore, considerably altered this portion of the instrument by making the screw portion, together with the fixed zero thread, movable in the other part, which might be aptly termed 'an eye-piece

adapter.' By this we secure the advantage of the double movable thread, without the additional error of the double movable thread, and this, moreover, without losing the convenienee of a fixed zero.

This enables you to span your object at equal distances on either side of the optic axis, without disturbing the centricity of the eye-piece. A guiding line has been added, because an error might creep in unless measurements are made with precisely the same portion of the wires.

'The divisions on the screw head have been made white on a black ground, on account of their being easier to read in a darkened room. A cap to protect the threads from dust and injury, &c., is provided, as the threads are no longer inclosed between the lenses, as in the Huyghenian form.

An iris diaphragm is placed below the threads and as close to them as possible.

In spanning the stage micrometer, it will be found better to take the readings from centre to centre of the lines, by doing which you avoid the diffraction which is always present at edges.

The measurement of all objects should be performed under a wide angled cone of illumination, so that the diffraction at the edges may be minimized as much as possible."

Two Readings of Scale 50 μ.

				diff.
A	1038	A	1038	0
B	35	B	36	+ 1
C	37	C	36	− 1
D	36	D	37	+ 1
E	30	E	29	− 1
F	37	F	37	0
G	65	G	64	− 1
H	32	H	32	0
I	29	I	30	+ 1
J	48	J	48	0
K	34	K	33	− 1
L	29	L	30	+ 1
M	40	M	40	0
N	45	N	45	0
O	38	O	37	− 1
P	44	P	43	− 1
Q	39	Q	39	0
R	40	R	41	+ 1
S	24	S	24	0
T	40	T	41	+ 1
20	760		760	0
	1038		1038	

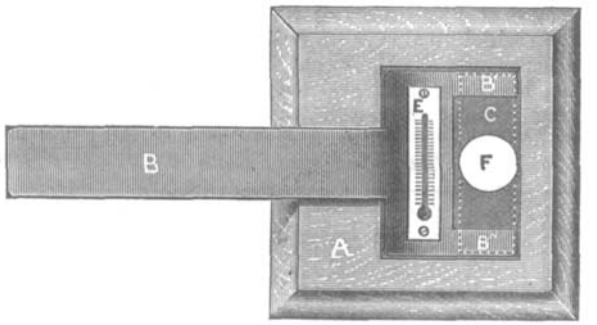
H H altered from 33 and 31 respectively.

An easily constructed Hot-stage.*—A very simple and convenient hot-stage was exhibited by Dr. Robert Reyburn at a recent meeting of

* Amer. Mon. Micr. Journ., xi. (1890) p. 1 (1 fig.).

the Washington Microscopical Society. This form is adapted from the more complicated and expensive forms used by microscopists, and is claimed to be especially useful from the fact that it can be made at a trifling cost by any one possessing a little mechanical skill. In fig. 56, A represents the wooden block or stage which is fastened upon the brass stage of the Microscope. A space is cut from the upper surface of this block, as shown by C, into which is fitted a piece of copper plate (B, B′, B″). A round hole is also cut at F, the opening of the brass stage, to allow of the illumination of the object to be examined. The slide is placed on the copper bed with its ends resting at B′ and B″, as indicated by the dotted lines. The heat is applied by a spirit-lamp at the end L of the copper plate B which

FIG. 56.



gradually transmits the heat by conduction to the slide. The temperature is registered by the thermometer E, which is screwed fast to the copper plate.

Application of Apertometer to the Microscope.*—Herr Kayser remarks that the narrowest perceptible distance of a wave-length stated by Fraunhofer and Nobert, is not the extreme limit which the newer Microscopes with oil-immersion systems have reached in the resolution of the structure of diatoms. The smallest recognizable distance ϵ approximates to the expression established theoretically by Helmholtz

$$\epsilon = \frac{\lambda}{2 \sin \alpha},$$

where λ denotes the wave-length and α the angle of divergence under which the extreme rays from the axis of the object fall upon the objective system. Since this angle can with an immersion lens be nearly a right angle, the numerical expression for the limit, taking $\lambda = 0{\cdot}00055$ mm. in the most general case, will amount to the half wave-length $0{\cdot}000275$ mm. According to the practical investigations of Abbe and Dippel, the resolving power of an objective system stands in very close relation to the magnitude of the angle of divergence. On this account makers are

* Schrift. der Naturforsch. Gesells. Danzig, vii. (1890) pp. xiii.–xvi.

obliged to have regard to the greatest possible angle of aperture or to the highest "numerical aperture" of Abbe, of which the expression is $a = n \sin u$ (where u is the refractive index). Accordingly they give in their price lists with dry systems the angle of aperture or the numerical aperture, and with immersion systems the latter. Whether these data correspond to the facts must be subjected to experiment. Herr Kayser received from a well-known firm an oil-immersion (1/16 in.), which he had required to be capable of resolving *Amphipleura pellucida* in oblique light. The system supplied did not answer to the requirements. The maker having ascribed the non-resolution to "badness of the preparation, defects in means of illumination, stand, &c.," it remained for a time doubtful whether these circumstances were really to blame. Herr Kayser was at that time engaged on the construction of apertometers. The apparatus resulting from his investigations, which serves for the examination of dry systems, has the following arrangement. Round a horizontal divided circle a vernier can be turned, and an upright, on which is fixed a Microscope directed horizontally, is set up in the centre. In front of the objective of the latter is a ring attached by a pin to the same upright. This ring can be rotated about the axis of the upright by means of side pieces which reach to the horizontal scale and carry a second vernier. With the plane of the ring at right angles to the axis of the Microscope, which passes through its centre, the reading on the second vernier is 90°, when the direction of the Microscope corresponds to the reading 0°. The system whose aperture is to be tested is placed in the ring. The Microscope is then displaced along its axis until the combined optical apparatus, which acts as a non-inverting telescope, shows the images distinctly. When by suitable turning of the whole apparatus the cross wires of the Microscope have been adjusted on an object not too near, the first vernier is displaced, without moving the second, both to right and left, until the image in each case just vanishes on the edge. The sum of the two angles read off is the angle of aperture. The angle thus given for an objective system, No. 7, of about 4 mm. focal length, was greater than the value given for it and found by the Abbe apertometer, in which the identity of an optician's systems of equal members is assumed. The author, attributing the magnification to his apparatus objective, tried the apertometer objective of Zeiss, specially made for the Abbe apertometer; but even with this the result remained unaffected.

The second apertometric apparatus constructed by Herr Kayser can be used for both dry and immersion systems. It consists simply of a glass plate of which one face is silvered and has scratched upon it a system of concentric circles which come into observation according to the dimensions of the apertures to be determined. The plate is laid on the stage with the silvered side downwards, and carries on its upper face in the middle of the rings, a small cover-glass, on the under side of which is a small mark. The Microscope containing the objective to be tested is first adjusted on this mark. Then without moving the body-tube the eye-piece is withdrawn, and again replaced in the tube when combined with the apertometer objective. The eye-piece is then adjusted so that the rings near the edge appear quite distinct; the extreme ring is counted, and if it does not exactly coincide with the edge, an estima-

tion in tenths of the following ring interval is made. A central portion of the silvering is removed and illumination by a mirror used in order to make the mark on the cover-glass visible. For the illumination of the rings, however, a white paper screen above the objective, and set obliquely to the incident light, is sufficient. The rings then appear dark on a white ground, and it is not necessary to have light incident from a mirror below. When an immersion system is to be tested, the observation is made in the same way except that, in this case, a drop of the liquid is first inserted between lens and cover-glass. To fix the diameter of the rings of this apparatus before they are actually scratched on the plate, a determination of the exact thickness of the glass plate and its refractive index must first be made. As found by the microscopical method, the first was 6·13 mm., the second = 1·525. The rings are arranged at intervals of 5/100 of the numerical aperture. The data, for example, for an aperture of 0·80 are as follows:—

$$0{\cdot}80 = 1{\cdot}525 \sin \chi,$$

whence the angle in the glass $\chi = 31^\circ\ 38'$, but

$$\tan \chi = \frac{r}{6{\cdot}13},$$

from which is deduced the radius of the ring in question $r = 3{\cdot}777$.

The angle of divergence α in air, since

$$n \sin \chi = \sin \alpha$$

is

$$\alpha = 53^\circ\ 7'.$$

The double amount 106° is therefore the angle of aperture corresponding to the numerical aperture 0·80 mm. The radii for the numerical apertures up to 1 would be as follows:—

0·80	3·777 mm.
0·85	4·115
0·90	4·481
0·95	4·881
1·00	5·324.

The plate contains in this way rings increasing in diameter up to the aperture

1·40	18·820 mm.

For greater distinctness, at certain intervals, two circles close together are drawn instead of one.

In testing the oil-immersion system previously referred to, the fifth reckoned from the ring corresponding to the aperture 0·80 fell on the edge of the field of view. It has, therefore, at most, the numerical aperture 1·00, whereas in the price list of the firm it was called 1·25. This was a great discrepancy, for if the system had really possessed the latter aperture, five more rings ought to have been seen. The numerical apertures necessary for the resolution of different diatoms are given in Dippel's text-book of general microscopy in the tables of comparison which have been established by exact scientific observations. On

reference to these tables, the data referring to 1·00 were found to be *Nitzschia curvula* and *Navicula rhomboides* (*Frustulia*) var. *saxonica* 36 striæ in 1/100 mm., while for the resolution of *Amphipleura pellucida* with 40–42 striæ, a system of 1·10–1·15 was found to be necessary.

Long before the use of the apertometric process, Herr Kayser had informed the maker of the system that he fixed the resolving power at 34 striæ from the fact that *Nitzschia curvula* was not resolved, and that *Frustulia* showed striæ first on the edges. The maker, ascribing the non-resolution to the mounting of the preparation, at the same time sent preparations which really were resolved. The striation of these, however, only amounted to 26 and 24 to 30 respectively, while *Amphipleura pellucida* was not forthcoming, because they were "at present not of good quality."

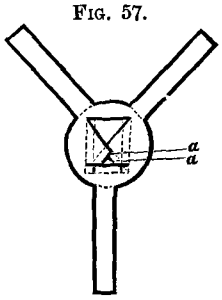
Dippel's work shows with what exactness the productions of microscopical forms can be apertometrically rated, in a way quite analogous to the determination of size by the scale. The action of an optician therefore who sells an objective system having a less aperture than it professes to have, must be compared to the behaviour of a tradesman who supplies goods deficient in quantity.

An advantage is now to be considered which the apertometer ring method possesses over that of Abbe. In the latter method a pointer is turned round on a polished glass cylinder until it appears to come on to the edge of the aperture. In this way the aperture is tested only in a certain diameter. By the author's method the whole range is seen at a glance, and any defects can also be noted. It is interesting that in the present dry system No. 7, the rings do not appear to be exactly concentric, but in a certain diametral direction on one edge there are broad intervals, on the opposite narrow ones, so that for the clear definition of the first, a further pressing in of the eye-piece is necessary. This asymmetry can be also recognized by the first method in the change in adjustment of the eye-piece, and out of the difference of the horizontal angle.

PLAXTON, J. W.—**A Camera Lucida for nothing.**

["The other day, after a morning's work, something went wrong with the prism of my camera lucida, and, do what I would, I could not bring it back to usefulness. At a loss for the moment, I cast about for a substitute, and in half-an-hour, with penknife and pencil, out of a piece of stiff paper and a square of thin glass, had turned out a fragile but efficient substitute for what is known in catalogues as 'Beale's Neutral Glass Reflector,' price 6*s*.

FIG. 57.



"This is how I did it—Describe a circle by standing the eye-piece of the Microscope on the paper and running a pencil round it; inscribe a square in the circle already drawn by drawing the pencil along the edges of the square of thin glass you intend to use; now lay down the diagonals of the square; draw three other lines within the square, each one parallel with a side of the square, and each, say 1/8 inch from the side; draw two other short lines (*a a* in the diagram, fig. 57) parallel to the diagonals.

Take the penknife and, following the continuous lines of the diagram, cut through the paper: you will have in paper what resembles a three-spoked

wheel without tire. The upper triangle of the four within the square falls away as useless; the lateral triangles open outwards, and stand at right angles with the plane of the circle; the little flanges on their lower edges are made by creasing the paper to support the thin glass. The base of the lower triangle answers the same purpose.

"Put the eye-piece in the Microscope, the circle of paper to the end of it; turn the spokes of the wheel back along the tube, and slip a tiny elastic band over them, or tie them with a thread; a little manipulation with the fingers, the thin glass is in place, and the thing is done.

"Need I say that any one can see that it would be almost as easy to use a piece of thin sheet brass or other metal as to use paper?"]

Journ. of Microscopy, III. (1890) pp. 40-1 (1 fig.).

(4) Photomicrography.

Some Experiences in Photomicrography.*—"More than a dozen years have now elapsed since I made my first photomicrograph; at that time the successful workers in this country could be counted on one's fingers, and the old messy wet process and expensive appliances were regarded as indispensable for good results. The development of amateur photography following the general introduction of dry plates could not fail to influence photomicrography, since few microscopists viewed with indifference the placing at their doors of a ready means of recording observations. The experience gained during ten years of continuous work, covering almost every class of subjects, with amplifications ranging from four to four thousand diameters, has, naturally, emphasized many facts; the possibility that a brief statement of these conclusions may aid younger workers in this field must be my apology for the egotism of these remarks.

While appreciating fully and endorsing heartily the efforts of those working with no greater responsibility than their individual enjoyment, it is rather to those seriously engaged in endeavouring to produce the best and highest class of this work, that these suggestions are offered.

The three essential conditions for success in photomicrography are:—(1) Satisfactory apparatus; (2) Good illumination; (3) Suitable preparations.

Satisfactory apparatus by no means implies elegant appliances, but adaptation to purpose; so that the Microscope be very solid and firm, and supplied with a substage to which a condenser can be attached and satisfactorily adjusted, and that the camera be of sufficient length, it matters little as to exact form or detail; for high powers the mechanical stage is a convenience, and for extreme amplifications (2000 and over) well nigh a necessity. The most complete photomicrographical outfit to be had is, undoubtedly, the one made by Zeiss, of Jena. . . . Of greater importance, however, is the quality of the objectives, for only those of the most perfect correction will stand the severe test of photography. While there are many others, which personal use has shown to answer well, my experience would lead me to select the following as being especially satisfactory:—3 in. Crouch, 1½ in. Beck, 3/4 in. Bausch and Lomb, 4/10 in. (BB) Zeiss, 1/4 in. "Photographical" Bausch and Lomb, 1/6 in. (DD) Zeiss, 1/12 in. oil-immersion apochromatic Zeiss. The 3 in. Crouch and the 4/10 in. (BB) Zeiss deserve especial mention,

* By George A. Piersol, M.D., of Philadelphia. Amer. Annual of Photography, 1890.

as for crisp definition over an entire perfectly flat field they are unsurpassed; for high amplifications the new apochromatic oil 1/12 Zeiss is superb.

The question, whether to receive the image directly from the objective on the plate, or to employ some means to project the image, has received of late much attention. While fully appreciating the theoretical objections to the direct image, I confess that for low and medium powers I continue to use it by preference, as the photographs so obtained fully equal in every respect any which I have ever seen made by the indirect mode. With high amplifications (1000 diameters and over), the conditions are greatly changed by the approach to the limit both of the shortness of the focus of the objective and of the length of camera which can be advantageously used; my experience leads me to adopt the 1/12 in. objective as the one, and not over four feet as the other limit, since any given high amplification, say 2000 diameters, can be more satisfactorily and more conveniently obtained with a superior 1/12 in. connection with suitable optical means to increase the initial magnifying power of the objective than with an unaided 1/25 lens and the plate removed to a great distance. Until quite recently the various amplifiers offered the best means of increasing the power of an objective, but the introduction of the "projection-oculars" of Zeiss has given us an accessory for this purpose far superior to the older devices. These projection-oculars resemble the ordinary microscopical oculars, or eye-pieces, only in general form and in name, being optically a projection-objective in connection with a collecting lens. The new oil-immersion apochromatic lenses, in combination with these projection-oculars, form, undoubtedly, the most efficient equipment for high-power work, and have but one drawback—their cost. It is, unfortunately, as true for high-power photography as for microscopical observation in general, that the best results are to be obtained only with fine, and necessarily expensive, optical appliances. If for the satisfactory study of the intimate structure of a cell, or of a micro-organism, the most improved immersion lenses are necessary, it is to be expected that for the successful photographing of the same, tools at least as good are needed. The complicated mechanical arrangement for controlling the focusing adjustments from a distance, may usually be replaced with advantage by the simple contrivance of cords and weights, devised by the writer more than a dozen years ago, which has been so generally adopted in this country; during the extended continued use of this little device, it has never been found wanting, responding perfectly to the severe demands of the highest amplifications. A modification for the coarse-adjustment, having pulleys and very heavy weights, serves equally well when very low (2 to 5 in.) lenses are used. A very stiff spring in the fine-adjustment may sometimes require increased friction to prevent the cord from slipping, the necessary traction being obtained by heavier weights, or by taking an extra turn of the cord about the milled head of the micrometer screw.

My conclusions regarding the second of the necessary conditions—good illumination—are briefly stated; after many experiments with various kinds of artificial illumination, and after the examination of innumerable specimens of the best work of acknowledged experts, while, of course, admitting that good photographs can be made, under suitable

conditions, by these means, yet I am fully pursuaded that sunlight is by all odds the best, and, for high powers, the only really satisfactory illumination by which to make photomicrographs that are satisfactory as photographs, as well as records of microscopical observations. That even by good lamplight fair impressions of objects under extreme magnification can be obtained, no one questions, but the negatives produced by such illumination seldom, if ever, possess the characteristics of a really good sunlight negative, where the sharpest details are combined with an exquisite softness and harmony of half-tones. That a photomicrograph should be a silhouette of deep shadows and chalky whites, is a proposition to which I could never subscribe. Sharpness and vigour are, of course, the first essentials in a photomicrograph, but there seems to be no reason that in such a picture all the qualities of a good photograph should not be represented. An almost identical opinion regarding the advantages of sunlight, has been reached by Dr. R. Zeiss,* after a most exhaustive series of experiments with artificial illuminations of all kinds, stimulated by the hope of finding a satisfactory substitute for sunlight, the uncertainty of which, during the greater part of the year, is even a greater inconvenience in Germany than with us.

The third condition for good work—suitable preparations—though last, is by no means least, for all apparatus and illumination avail but little when proper preparations are wanting. Thanks to our present microscopical technique, these are readily obtained, since extremely thin and well-stained preparations of vegetal and animal tissues are now matters of everyday production. The thinness with which sections are now usually cut (·005–·01 mm.) often renders them, when stained with the staple carmine dyes, too actinically transparent to photograph well with very low powers. The interposition of some ray-filter readily overcomes this; during the last three years a screen of yellowish-green glass has been in constant use, with the most satisfactory results, yielding plucky pictures of objects entirely too transparent to produce sufficient contrasts in the negatives; the exposure, however, is increased about three to five times, but this, even when thus lengthened, seldom exceeds 20–25 seconds, on Carbutt's "B 12" plates. Where great differences of colour are present in the same preparation, or where certain unfavourable tints, as deep brown, prevail, the orthochromatic plates offer decided advantages; for, however, ordinary preparations with but one stain, the colour-screens, when judiciously selected, will yield equally good pictures, with a gain in economy, convenience, and certainty. The modified hæmatoxylin stains, producing browns and slate-blues, are very valuable for special purposes, but require some considerable technical experience for their successful production.

What has been written may appear to discourage the undertaking of this most fascinating branch of photography, where the primary object of instructive entertainment does not warrant the acquisition of the class of appliances above recommended; this should not be so, as the full force of these suggestions applies only to those whose work in this line necessitates the use of the higher amplifications, with the view of producing the highest possible results."

* 'Special-Catalog über Apparate für Mikrophotographie,' Jena, 1888.

To the amateur, who has been using but lamplight for his exposures, it is suggested that he avail himself of some bright "off-day" to give sunlight a trial. If the mirror of the Microscope be of good size, it will be only necessary to make an arm on which to support the removed mirror outside some southerly exposed window, since it is desirable to have much more distance between the mirror and the stage than would be possible were the mirror attached in its usual place. Where the Microscope mirror is too small to be satisfactorily used, a rectangular wood-framed looking-glass is readily mounted with the aid of a few strips of wood, so as to turn about both axes.

The rays from the plane side of the mirror are passed through a condensing lens (of 8–10 in. focus, if possible), so placed that they are brought to a focus before reaching the plane of the object. The exact position of the condensing lens is a matter of experience; usually, however, the most favourable illumination is obtained at that point where the field is still *uniformly* illuminated, just before the rays form an image of the source of light; the nearer the rays are focused, the less disturbance from diffraction rings. Ordinary objectives will require the employment of monochromatic light—produced either by a deep blue solution of ammonio-sulphate of copper, or by the green glass screen already mentioned—since the optical and actinic foci do not usually coincide. Powers up to the 3/4 in. will require no further condenser; with the 1/4 or 1/6 objectives, the low power (1 or 3/4 in.) serves with advantage as an achromatic condenser, when attached to the substage. The Abbe condenser, although so important for refined microscopical investigation, is not adapted to photography unless a very wide cone of light is desired, which, for the majority of preparations, is a decided disadvantage; a low-power objective, used as a condenser, will generally be found more satisfactory than the Abbe with a small diaphragm.

The simple apparatus indicated, when properly handled, will produce excellent work with such powers as the amateur is likely to employ; focusing the image by the monochromatic light, and avoiding over-exposure, being the points especially requiring experience. When it is remembered that seconds, with very slow plates, usually suffice for the minutes with rapid ones of an exposure by lamplight, the intensity of the actinic power of the sunlight will be somewhat appreciated. Some simple arrangement, by which the rays from the mirror may be cut off with sufficient rapidity, will suggest itself; an effective one is a small shutter, turning at one end on a screw and covering a circular opening in a board, through which the rays from the mirror pass; the rapidity with which the sun's image from a fixed mirror becomes decentered necessitates a readjustment of the light just before each exposure, but the patience thus exercised will be more than repaid in the character of the resulting negatives.

Microphotographs of Wood Sections.—An interesting communication on this subject was recently made by MM. Thil and Thouronde to the French Photographic Society. Microphotographs to the number of about four hundred were executed to the order of the Minister of Agriculture. M. Thil, Inspector of Government Forests, has, in very precise language, pointed out the reach of this application of photography, which permits of the classification of woods in families

and species, thanks to the comparison alone of the intimate structure of the fibres and cellular network. By this means we are enabled, with the help of simply thin cuttings, to give, so to say, a complete anatomy of each species, and to notice easily the essential differences which exist between woods of different species, although belonging to the same family; all the more, therefore, can we recognize classification in families. Microphotographic pictures, projected by the lantern, served to demonstrate clearly the truth of the propositions affirmed. This is a new example of the numerous services that photography may render to the sciences.

The Coloured Screen in Photomicrography.*—The following is an abstract of a paper by Professor Romyn Hitchcock :—

An ordinary gelatino-bromide plate is sensitive to the spectrum of sunlight from a point between the Fraunhofer lines E and F to about K. The maximum photographic action is about G. By considerably prolonging the time of exposure the limit of photographic action at the red end of the spectrum is greatly extended. In practice the light below the green of the spectrum may be regarded as quite inactive when we take photographs with ordinary plates.

By introducing a coloured screen—a plate of yellow glass for example —in the path of the light, we may absorb the more active rays, and prolong the time of exposure until the yellow rays have time to act upon the sensitive plate. In practice, however, it is found that there are two difficulties about this method of procedure; first, in obtaining a satisfactory screen, and second, in the long exposure necessary when working with the comparatively inactive rays.

With colour-sensitive plates, such as are now in general use abroad and gradually being introduced in this country, the range of photographic action towards the red is greatly extended. With such plates the yellow screen can be used with great advantage.

A few years since it was customary to work with monochromatic blue light in photomicrography, and the ammonio-sulphate of copper blue cell was much in use. When colour-sensitive plates were introduced yellow screens took the place of blue, because it was found that many specimens had yellow and red and brown parts which were not well photographed with blue light.

The colour and thickness of the screen both require attention. If it be too thin the blue light is not sufficiently cut off. In particular cases an almost monochromatic yellow light is desirable, as when it is desired to obtain sharp outlines of deeply stained objects regardless of structural details. But generally a rather broader spectrum range is desirable, for the light employed should correspond to the different colours or shades of colour of the object. It is owing to neglect of this consideration that we often see photomicrographs which are mere silhouettes, while the objects show much more structure to the eye. This is frequently observed in photographs of such structures as the tongue and sting of a bee, and legs of insects. In other preparations, in which the colour is a stain, brown or red for example, the fault lies partly in the exposure, which, in many cases, is insufficient to give more than

* Amer. Mon. Micr. Journ., xi. (1890) p. 8.

outlines and blank interiors. This is frequently noticeable in photographs of bacteria.

By a proper choice of a screen, if a screen is required, a photograph should show any object as clearly as we can see it in the Microscope.

Colour-sensitive plates may be said to be indispensable in the photography of rock-sections with polarized light.

The yellow solution devised by Professor Zettnow, of Berlin, is used with much favour by many workers. It is composed as follows:—Copper sulphate, 175 grm.; potassic bichromate, 17 grm.; water, 1000 ccm.

The true function of the colour-screen should be to give definition and detail, not to increase contrast between the object and the field, as many observers seem to believe.

(5) Microscopical Optics and Manipulation.

Amplification in Micrometry.*—My attention has quite recently been drawn to this subject in connection with the celebrated Dr. Cronin case. It may be taken for granted that one cannot measure what he cannot see. But how high an amplification is necessary in a given case is a matter of much importance. In the measurement of blood-corpuscles in medico-legal cases the late Dr. Richardson advocated the use of a very high power, viz. a 1/25 or 1/50 objective. In my own measurements of blood-corpuscles I have, out of respect to authority, always used a high power, from 1500 to 1800 diameters. Recent experience has, however, qualified my views upon the subject, and in the case of the comparison of the ultimate subdivisions of a micrometer, ruled on metal, I am now of opinion that practically the same result may be obtained by the use of a 1/4 objective as with a 1/18 or 1/25.

In December 1885, I commenced the investigation of the 1/100 mm. spaces of "Centimeter A"; but was unable to finish it. Two series of measurements were then made with a Bausch and Lomb opaque illuminating objective, and a Bulloch filar micrometer. Recently I have measured the same spaces with a Spencer 1/10 and 1/25, and with a Zeiss 1/18. The results of these measurements are given in the table below, each correction being the mean of from three to twelve readings of the filar micrometer at each end of the measured space.

It will be observed that the agreement between the several series of the writer, and the results obtained by Prof. Hilgard is quite close, the discrepancy being practically insensible.

Provided the amplification is sufficient to render the object to be measured of a sensible size, and to render the difference between the sizes of two objects visible, my own judgment is that little, if anything, is gained by the use of a power so high as to impair the definition, even though such impairment be but slight. Quite as much, in other words, is lost by impairment of definition as is gained by increase of amplification. The practical conclusion then is that no higher power should be used than is consistent with perfect definition.

Diffraction Rings and Diffraction Spectra.—There appears to be still some confusion between the diffraction "spectra" of the Abbe theory and the diffraction bands or fringes and spurious lines seen

* By Hon. Marshall D. Ewell, LL.D.

surrounding the outlines of all objects in the field of the Microscope, when the illumination is obtained by somewhat narrow but sufficiently bright beams of light, especially with high powers or deep eye-pieces.

The latter are true diffraction bands, originating from the diffraction of the light at the object, but the difference between the two phenomena is that the spectra represent the diffraction effect of the object at a very distant plane, conjugate to the posterior focus of the objective, whilst the "bands" or "fringes" show the diffraction effect of the same objects in a plane close by, i. e. in the neighbourhood of the objects themselves. Nägeli and Schwendener, it is true, deny that these fringes are diffraction phenomena, and explain them as interference phenomena in a somewhat complicated manner, but Prof. Abbe considers that he has established the incorrectness of their views on this point, except so far as they assert that the phenomena cannot be due to the diffraction effect of the lens opening, as had previously been assumed by Helmholtz and others.

(6) Miscellaneous.

The 300th Jubilee of the Microscope.*—"B. C." writes:—Natural science enters this year on a memorable anniversary, the 300th Jubilee of the Microscope, one of the most powerful of its resources. To this instrument is due in great measure the wonderful impulse given to science in the second half of this century. The importance to which the Microscope has attained in scientific investigations is well known. It has become an absolutely indispensable instrument to the zoologist and botanist, to the mineralogist and geologist, to the astronomer and the physician. The Microscope has effected a complete revolution, and has diverted the direction of study into the most varied channels. In fact it has created a new method of research, such as histology. On the healing art the Microscope has exercised a most beneficent influence; for while it explained the changes undergone by the finest tissues in the various diseases—it was on microscopic observation alone that Virchow founded his renowned system of cellular pathology—it pointed out at the same time the means of healing them. The Microscope has also been of wonderful service in technical matters. Before attaining its present high degree of perfection, the Microscope had to pass through a number of intermediate stages which it is of great interest to look back upon on this its 300th jubilee. . . .

It is strange how slowly the Microscope found its way into learned circles. It was only when Leeuwenhoek had by its aid discovered the infusoria that it became generally used in the scientific investigations of anatomists and physiologists. What it has accomplished since that time constitutes the glory of the natural sciences. The Microscope soon passed from the workshops of the spectacle-makers to those of the optician, by whose skill it has undergone, little by little, numerous changes, corrections, and improvements. Not to mention all of these, it will suffice to point out the arrangement of the transmitted light (1685), of the reflecting illuminating mirror (1715), and the use of achromatic and aplanatic objective lenses (1824). In more recent times the Microscope has received further improvements, which have cast into the shade all conceivable expectation; and unless appearances deceive us the finer

* Central-Ztg. f. Optik u. Mechanik, xi. (1890) pp. 69–70.

mechanics of Microscope construction have not yet reached the limit of their capabilities. The latest acquisition of medical science, the bacteria, has put the greatest demands on the Microscope, and reveals to this instrument the deepest secrets of nature. Let it be the aim of science to gather in a still richer harvest by the aid of the Microscope!

The Microscope banished.—The following appears in the *Daily News* of the 9th April:—"An interesting paper by Mr. Bothamley in *The Photographic Quarterly* reminds us of the important part now played in education by the optical lantern which in the memory of so many among us was a mere toy for the entertainment of juvenile parties. The initiation and growth of the system is mainly due to Professor Miall, of the Yorkshire College, Leeds, in which important institution almost every department has its lantern, and such widely different subjects as biology and engineering, ancient history and textile industries are alike illustrated by this convenient means. In the biology lectures *the lantern is said to have well nigh banished the Microscope, thereby effecting a great saving both in cost and time* (!) The production of lantern slides is found to be most easily and rapidly done by photography. Original objects, drawings, large photographs, illustrations in text-books, can all be reproduced in the same way. At the Yorkshire College the number of slides required by the various departments is stated to be so large that the whole time of a special photographic assistant is occupied with their production, although the work is much facilitated by the ingenious copying camera devised by Professors Barr and Stroud. But perhaps the most remarkable fact in connection with this subject is Professor Miall's discovery of how the lantern may be used in illustrating lectures in a room illuminated by daylight."

Miss V. A. Latham, F.R.M.S.* — This lady has recently been elected to the chair of Demonstrator in Pathology in the University of Michigan. Professor Latham is the first lady who has held any office in the Medical Department of the University, and has our congratulations and best wishes for her success.

β. Technique.†

BÖHM, A., U. A. OPPEL.—**Taschenbuch der mikroskopischen Technik.** (Handbook of microscopical technique.)
München (Oldenbourg), 1890, sm. 8vo, 155 pp.

GORONOWITSCH, —.—**Kurze Uebersicht über die Fortschritte in der mikroskopischen Technik im Jahre 1888.** (Short review of the progress in microscopical technique in 1888.) *Medizinsk. Obozrenije*, 1889, No. 8 (Russian).

KAHLDEN, C. VON.—**Technik der histologischen Untersuchung pathologisch-anatomischer Präparate.** Für Studirende und Aerzte. Ergänzungsheft zu Dr. E. Ziegler's Lehrbuch der allgemeinen und speciellen pathologischen Anatomie. (Technique of the histological examination of pathological-anatomical preparations. A supplement to Dr. E. Ziegler's *Handbook for the use of Students and Physicians.*) 6th ed., Jena (Fischer), 1889.

POLI, A.—**Note di microtecnica.** (Notes on microtechnique.)
Malpighia, III. (1889) June, August, December.

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* Amer. Mon. Micr. Journ., xi. (1890) p. 10.

† 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.

(1) Collecting Objects, including Culture Processes.

Procuring and Preparing Protista found in the Stomachs of Ruminants.*—To obtain Protista from the stomachs of oxen, says Dr. A. Fiorentini, it is merely necessary to open that viscus with a knife, and gather some of the gastric juice in test-tubes. In order to keep the animals alive it is advisable to keep the tubes immersed in water at a temperature of 30°–35°. To examine these Protozoa alive, it is necessary to make use of Schultze's or Ranvier's hot-stage, so that the slide may be kept at 35°. But the following method has the advantage of simplicity. Heat the slide over a spirit-lamp until it becomes warm. Then place thereon a drop of the fluid containing the animals to be examined, and cover with the cover-glass. Next with a pipette take some boiling water and drop it in lines on the slide, taking care, however, that it does not mix with the fluid under the cover-glass. This device will keep the preparation warm sufficiently long to examine the Protozoa alive. When cold a new preparation must be made.

For fixing the animals, the author used a 1 per cent. osmic, and for staining the nuclei and nucleoli fuchsin, alum-carmine, and alum-cochineal. Glycerin and Canada balsam were used for clearing up the preparations when osmic acid had blackened them or made them obscure.

Useful Collecting Device.†—Mr. J. Walker finding his collecting bottle, a modified Wright, somewhat cumbersome, "decided to use a smaller bottle, and have the strainer (I use bolting silk 10,000 to the inch) outside instead of inside. I therefore procured a bottle holding about 4 oz. A square bottle with a wide mouth is preferable, though a round one will answer well. I bored four holes opposite each other, 1 in. above the bottom and about 3/8 in. in diameter, and enlarged the openings in a direction parallel with the length of the bottle, until within an inch of the neck. Over these four oblong apertures I cemented fine bolting silk or other desirable material with shellac, and when dry, the bottle was ready for use. To those not having the tools needed for drilling glass, I would recommend a small tin can or box, such as that in which Colman's mustard is sold, or the common round pepper-box obtainable from the grocery stores, the lid making a good coarse strainer.

In working with it, the currents of water passing through the meshes of the strainer will cause fine debris to collect on the inside, which in this case is easily kept clean with a small brush, a piece of wood, or a stalk of grass. The concentrated material will be found at the bottom of the vessel, and can be transferred to another small bottle carried for the purpose."

Collecting-bottle for Rotifers.‡—Mr. A. Pell remarks, "Here is the 'boss' collecting implement at last. Take one of the new lard bottles which hold a quart, the mouth being about 4 in. across, with a metal cover that screws to the neck, and a handle by which it is readily carried. Make a tube of muslin or of linen, in any desirable

* Journ. de Micrographie, xiv. (1890) pp. 15–6.

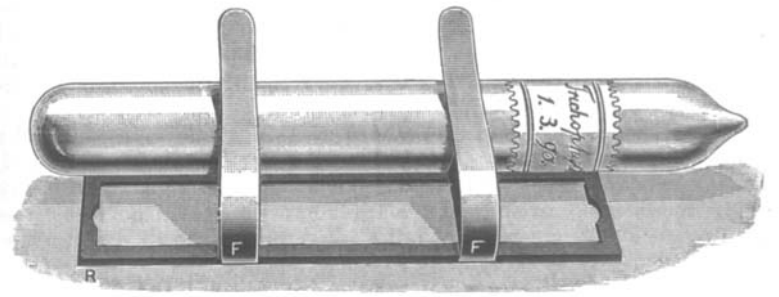
† The Microscope, ix. (1889) pp. 372–4.

‡ Op. c., x. (1890) p. 151.

fineness, and about 2 feet long, 4 in. in diameter at one end, and 2 at the other. Fasten a tin ring to the small end and attach the large end to the mouth of the bottle. Then put on your rubber boots and go to the pond. There pour the water into the small end of the muslin tube, holding it up for that purpose, the bottle hanging below. It will rapidly strain out the Rotifers, &c., which will finally get down into the bottle, and as the muslin tube has so large a surface the water will run through quickly, all solid matters collecting in the bottle. Less is lost by the use of the muslin tube than by a funnel-shaped strainer, and the cloth will not become clogged."

Test-tube Holder for Microscopical Investigations.*—Dr. D. von Sehlen has invented a test-tube holder, the advantages of which are mainly its stability and simplicity. Hence it will be found of great use in the cultivation of the various forms of Fungi, and also for photo-

FIG. 58.



graphic purposes. The apparatus consists of a flat oblong frame R which supports two uprights, placed equidistant from the ends of the frame. In these uprights a triangular piece is cut out in order to put the test-tube in, and the latter is kept in position by the two spring-clamps F. The distance between the two spring-clamps is enough to allow sufficient space for the objective to work in, and the length of the frame such that it is easily clamped to the Microscope-stage. It is hardly necessary to explain that the test-tube is easily moved round its short axis, and pushed up and down, so that when on the Microscope-stage it is easily illuminated from below.

Preparation of Nutritive Agar.†—Dr. V. A. Moore writes:—"The extent to which nutritive agar is employed in the cultivation of Bacteria renders it of much importance that its method of preparation should be made as perfect as possible. When it is prepared after the method recommended in works on bacteriology (which is practically the same as that first formulated by Koch for the preparation of solid culture media), a medium is obtained that favours the growth of most germs. In this respect the method is desirable, but in regard to the other

* Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 17–20 (2 figs.).
† Amer. Mon. Micr. Journ., xi. (1890) pp. 115–7.

requisites of a satisfactory solid medium it is quite deficient. The objections to the method with reference both to the process itself and the character of the resultant agar are three in number. (1) The difficulties attending the filtration of the agar. This process alone often requires a very considerable length of time besides the use of a hot filtering apparatus that must be provided especially for this purpose. (2) The presence in the sterile agar of a flocculent precipitate that is invariably thrown down during the process of its sterilization, and which greatly interferes with its usefulness, especially in making roll and plate cultures. (3) The variation in the consistency of the agar. It is impossible to obtain this material of the same consistency, as the agar is only partially dissolved, even after long boiling, in the simple beef-infusion. The coagulation of the albumen ensheaths the stems of agar, floats them to the surface where they remain imbedded in the firm, albuminous coagulum. This property of the agar is worthy of consideration, for with the varying consistency of the medium a consequent change follows in the character of the growth of most germs.

For the purpose of securing a process for the preparation of nutritive agar that was free from the above mentioned difficulties I have reviewed carefully the method of Jacobi,* Von Freudenreich,† and Cheesman,‡ in all of which I found difficulties that were equally as objectionable as those possessed by the original method.

The use of a solution of beef-extract in distilled water, instead of the simple beef-infusion made directly from the fresh meat, was also tried, but the agar thus prepared did not favour as vigorous a growth of many germs as when prepared from the fresh meat-infusion. So feeble was the growth of many germs upon this agar that the method was abandoned, although very satisfactory in other respects.

In the course of this experimental work it was found that when the stems of agar were cut into small pieces and boiled in a fluid containing no coagulate material, that it was entirely broken up and the soluble portion dissolved. The insoluble particles that remained suspended in the liquid were easily and completely removed by the addition of egg albumen, and subsequent boiling and filtering. From these facts a method for the preparation of nutritive agar was derived, which consists in first preparing the neutralized beef-infusion-peptone, and thus getting rid of all coagulable material before the agar is added. This process is effective in greatly diminishing the time and attention required for the preparation of this medium. The medium can always be made of the same consistency, as all of the agar that is added is dissolved. It remains free from precipitates when sterilized, and its nutritive qualities are as favourable to bacterial growth as when it is prepared after the original method.

(1) *The preparation of the beef-infusion-peptone.*—The method of preparing this liquid is practically the same as that already in use in most laboratories. Finely chopped or ground beef (freed from fat) is macerated in distilled water for from 12 to 18 hours in a cool place. The distilled water is added in the proportion of 200 ccm. to each

* Centralbl. f. Bacteriol. u. Parasitenk., iii. (1888) p. 538. † T. c., p. 797.
‡ American Naturalist, xxii. (1888) p. 472.

100 grams of beef. On the following day the liquid is separated from the meat by straining it through a coarse linen. The simple beef-infusion thus obtained should be equal in quantity to the amount of water added; if it is not the deficiency can be restored by the addition of distilled water. To the beef-infusion is added 1 per cent. peptone, 1/2 per cent. sodium chloride; and if it is desirable to make it alkaline, a sufficient quantity of a normal solution of sodium carbonate to give it a weak alkaline reaction. The liquid is then boiled for thirty minutes in a water-bath, cooled, filtered, and distributed in Erlenmeyer flasks plugged with cotton-wool. If only a small quantity of agar is to be made at once, 250 ccm. is found to be a very convenient quantity to put in each flask. It is then sterilized by boiling for one hour each day for three consecutive days. It need not be sterilized if it is desirable to prepare the agar at once. As the beef-infusion-peptone is also employed as a liquid medium in the cultivation of bacteria, very little time is lost in preparing an extra quantity of this liquid to be used in making the agar.

(2) *The preparation of the agar.*—To an Erlenmeyer flask (a glass beaker or agate or iron vessel may be used) containing beef-infusion-peptone, as prepared above, 1 per cent. of *very finely chopped* agar is added. The flask is then placed in a water-bath and boiled vigorously for two hours. At the end of that time the agar is dissolved, and the liquid is allowed to cool. When a temperature of 40–45° C. is reached, the white of egg is added in the proportion of one egg to 250 ccm. of the liquid. After the albumen is *thoroughly* mixed with the liquid agar it is returned to the water-bath and again boiled for two hours. It is of much importance that the albumen is evenly distributed throughout the mass before it is coagulated. It is now ready to be filtered. The egg albumen is coagulated in very firm masses, leaving the liquid perfectly clear. The coagulum is removed by filtering the liquid through fine Japanese filter-paper or a layer of absorbent cotton, as a 1 per cent. solution of the agar does not pass readily through ordinary filter-paper. Should a weaker solution of the agar (1/2 to 3/4 per cent.) be desired, its filtration can be accomplished by the ordinary method. A hot filtering apparatus is not necessary. The clear filtration is now ready for distribution in sterile cotton-plugged tubes.

The agar is sterilized by discontinuous boiling in a closed water-bath for three consecutive days. If small tubes have been used containing not more than 7 ccm. each, five minutes' boiling each day is sufficient. If larger tubes are used, they should be boiled for a longer time. Or it may be sterilized by steaming each day for from five to ten minutes after the agar has become liquefied for the same number of days. After its sterility has been tested by allowing it to stand in an incubator for several days, it is ready to be stored until required for use. It has been customary in this laboratory, in order to prevent the evaporation of the agar by long standing, to dip the lower end of the cotton-plugs in hot sterilized paraffin, and to store the tubes in a cool, moist chamber."

(2) Preparing Objects.

Preparation of Crustacea.*—Dr. O. vom Rath gives an account of the method he adopted in his investigation into the structure of the Cymothoid Crustacean *Anilocra mediterranea*. The heads were cut off with a sharp pair of scissors and immediately placed in picric-nitric acid, picric-sulphuric acid, warmed absolute alcohol or chrom-osmic-acetic acid; the first of these reagents gives especially good results. The hardened heads were stained *in toto* in alum-carmine or borax-carmine. Paul Meyer was quite right in urging that the mere preservation in alcohol of Crustacea or other Arthropods with a strong chitinous membrane is quite insufficient.

Modes of Studying Segmental Organs of Hirudinea.†—M. H. Bolsius did not learn much by dissecting out the segmental organs and mounting them entire. It is better to cut sections of the entire animal, or, when it is large, of parts. Transverse, vertical, longitudinal, or horizontal longitudinal sections should be made. To prevent contraction of the body, large specimens should be anæsthetized before being killed. Small specimens should be placed in a 1 per cent. (or even weaker) solution of chromic acid. Passable results in the way of fixation were obtained by bichromate of potash, but bichloride of mercury is much more efficient. A saturated aqueous solution or Gilson's liquid may be used. In either case small individuals are placed in them for 15 to 30 minutes; larger pieces must remain a proportionately longer time. Excellent preparations were also obtained with a 2 per cent. solution of nitrate of silver; in this case staining reagents were not used, but with the others a picro-alum-carmine, the formula for which has not yet been published, but which is used at Louvain, was found to give excellent results.

Mode of Investigating Hydra fusca.‡—Herr K. C. Schneider recognizes that it is only possible to study the nervous system of Hydroids by maceration-processes. It is scarcely possible to recognize in sections the cell-boundaries of the ectoderm, to say nothing of distinguishing them from the separate subepithelial elements. The structure of the cells is considerably affected by the use of paraffin. As a maceration-medium, the author first used pure acetic acid from 1 to 10 per cent; but as this caused deformation of the elements, chloride of sodium was used, and was followed by various strengths and quantities of osmic acid. After some experiments, a mixture of one part 0·02 per cent. osmic acid with four parts 5 per cent. acetic acid was found to give excellent results. Pure osmic acid was found to give very different results from the mixture of osmic and acetic acids. Animals placed for eight days or more in glycerin were very useful in the study of the nervous system. Picrocarmine was found to be the best staining medium, but Beale's carmine and safranin were also of use.

Microscopical Sections of Tooth and Bone.§—It was with great satisfaction that we read Mr. J. Howard Mummery's notes on the prepa-

* Zool. Anzeig., xiii. (1890) p. 232. † La Cellule, iv. (1890) pp. 374-6.
‡ Arch. f. Mikr. Anat., xxxv. (1890) pp. 322-3.
§ Trans. Odontol. Soc. Great Britain, xxii. (1890) p. 207.

ration of microscopical sections of tooth and bone, in which he gives an account of some new and important discoveries in the structure of these tissues, for it was from this Journal,* he tells us, that he obtained an account of Dr. L. A. Weil's method of carrying out the balsam process. "I prepared," says Mr. Mummery, "some sections according to these directions, and was so pleased with the results that I have since cut nearly two hundred specimens in this way." It should not be forgotten that this portion of the Journal is of great assistance to those who, like Mr. Mummery, have little time for searching the literature of microscopical technique.

Preparing Sections of Teeth.† — Mr. W. A. Hopewell-Smith remarks:—

"(1) The most satisfactory method, in my opinion, of preparing sections showing odontoblasts *in situ* is as follows:—The jaw, preferably the lower, of an embryonic mammal, such as kitten or pup, taken while still in a fresh condition, is carefully stripped of all the tissues covering it, except the oral epithelium and flange of gum, and is placed in the usual standardized solution of Müller's fluid, in order to harden its soft structures, the volume of fluid being about twenty or thirty times the bulk of the immersed tissue. The fluid must be changed every day for four or five days, and then every third or fourth day. The hardening process is to be completed by removing the specimen—which has remained in the Müller's for a fortnight—to alcohol or rectified spirit; and this is to be renewed occasionally until all the colouring matter has disappeared from the specimen and fluid. Vertical sections are then cut by means of a thin sharp knife, and these placed longitudinally on the stage of a Cathcart or Williams freezing microtome, and cut in the ordinary way. Best results are obtained from sections in the canine and bicuspid regions, as here the parts are less likely to be disturbed in the manipulations with the microtome. Imbedding in paraffin and wax, or celluloidin, is of little service. The advantages claimed for this method are:—(*a*) The simplicity of its performance. It will be seen that the hard tissues are not softened by any decalcifying agent, which would materially affect the delicate soft tissues. The knife cuts quite easily the thin cap of semi-calcified dentine and bone, and the elements of the pulp are in no way disturbed in their relation to each other. (*b*) The odontoblasts are of large size, and easily observable at this period, as their formation of dentinal fibrils is at its highest stage of development. They can be isolated, if thought necessary, by separating with the point of a needle from the surface of the dentine papilla the cap of dentine to which in places they adhere. (*c*) This method affects little, if at all, the relative positions of dentine, odontoblasts and pulp; and I have found it to be extremely successful.

(2) I should advise your correspondent not to grind down sections of teeth of fishes *in situ;* but to decalcify the jaw and teeth with a 5 per cent. solution of chromic acid or 10 per cent. solution of HCl. After sections have been cut and stained they should be washed well in distilled water, dehydrated for three minutes in absolute alcohol, "cleared" in oil of cloves or xanthol, and mounted in Canada balsam.

* 1888, p. 1042. † Journ. Brit. Dental. Assoc., xi. (1890) pp. 310–2.

Carmine is the best stain for fishes' teeth. If it is used, however, it is necessary before transferring to distilled water to pass the section quickly through weak $HC_2H_3O_2$ as this "fixes" the stain. If gold chloride is used the specimens must be mounted in glycerin-jelly. . . .

(5) It is unnecessary to cut sections of enamel to demonstrate the prisms. After having softened enamel by immersion in 10 per cent. solution of HCl, remove by means of a needle-point or fine brush a small portion to a slide; put a drop of normal salt solution on to the top of the enamel, and press down cover-glass. Then run a solution of carmine or orange-rubine beneath the cover-glass, and draw off the excess with a little blotting-paper. Wash the stain away further by irrigation with weak HCl, or $HC_2H_3O_2$, and mount in this solution or acidified glycerin after Beale's plan.

Examining Nuclei of White Blood-corpuscles.*—The ordinary notion about white corpuscles, viz. that the majority are polynucleated, is, says M. Mayet, quite erroneous. By this the author does not mean that polynucleated corpuscles are not demonstrable, but that this condition is extremely rare.

To ascertain exactly the shape of the nucleus, glacial acetic acid must be intimately mixed with the blood in the proportion of three to one.

By this means the red corpuscles are rendered almost invisible, while the extra-nuclear part of the white is more or less dissolved, so that the nuclei are isolated and become very visible.

The nucleus then is found to be of very variable shape, and it is owing to this irregularity that various optical effects are produced, so as to give the appearance of more than one nucleus. The nucleoli are always multiple, there being one for each swelling of the nucleus.

When a white corpuscle is really polynucleated, it is just in the act of division, nucleus and extra-nuclear plasma as well, but this condition is rare.

Studies in Cell-division.†—Prof. D. H. Campbell recommends the following subjects as specially well adapted for showing the various stages of division in the plant-cell, and its modifications; the paper is accompanied by very good figures:—For cell-division where there is no definite nucleus—*Nostoc*. For division of a multinucleate cell, and division of the nucleus independently of cell-division—*Cladophora*. For cell-division accompanied by the division of the single nucleus—*Spirogyra*. If exposed to cold during the night, and brought into the laboratory in the morning, some of the cells will probably begin to divide almost immediately. An interesting modification of the process is shown by many desmids. For following the process in the living cell—the hairs on the filaments of *Tradescantia virginica*. It is well shown by removing the stamens from the young buds, and mounting the attached hairs in water or in a 3 per cent. solution of sugar. They may be stained without killing them by a weak aqueous solution of methyl-violet, dahlia, or mauvein. For easy demonstration of the process of karyokinesis—the final divisions of the pollen-mother-cells,

* Comptes Rendus, cx. (1890) pp. 475-7.

† Bull. Torrey Bot. Club, xvii. (1890) pp. 113-21 (2 pls.).

especially of Monocotyledons as *Allium canadense*, or among Dycotyledons *Podophyllum peltatum*. The latter is especially favourable for showing the early stages, because of the small number (about ten) of the nuclear segments.

Dehydration and clearing up of Algæ.*—The following method, described by Dr. E. Overton, neither requires complicated apparatus nor demands a great expenditure of time, in obtaining a result more favourable than is usually expected when dealing with such delicate objects as Algæ, which shrivel or crumple up when transferred from one reagent to another.

The object, previously fixed and stained, is placed in a not too large quantity of 10 per cent. glycerin. Here it remains in an open vessel until the glycerin has given off nearly all its water. The objects are then transferred to absolute alcohol. Their further treatment depends on the nature of the clarifying medium. If turpentine, oil of cloves, or the like is to be employed, the object should be placed in a watch-glass, containing a 10 per cent. solution of the oil in absolute alcohol. The watch-glass is placed in a large covered vessel, on the floor of which are some pieces of calcium chloride to absorb the alcohol. In this way the objects are gradually impregnated with the pure oil, whereupon they may be transferred to dilute balsam. If before the objects be placed in the ethereal oil and alcohol mixture, they be passed through chloroform, this step will avoid the too great extraction of the staining by the spirit.

Should xylol be preferred for clearing up, then in the larger vessel pure xylol is placed as well as in the watch-glass. By a process of diffusion the inner vessel will ultimately contain almost pure xylol. By means of this method the most delicate algæ may be mounted in balsam without crumpling.

Amplification required to show Tubercle Bacilli.†—When properly stained and prepared, the bacillus tuberculi can be readily recognized with a good 1/5 objective and a 2-in. eye-piece, normal tube-length, or, roughly speaking, an amplification of 250 diameters. We do not think that it could be done much below this amplification, though the sharpness of vision of the observer, his acquaintance with the object, and the excellence of his objective would be important factors in settling the question. A 1/4 objective with a 2-in. eye-piece, normal tube-length, gives an approximate amplification of 200 diameters.

To be seen and diagnosed for certain, the bacillus tuberculi in urine or water must be prepared for examination by following the well-known technique in such cases (fixing, staining, bleaching, and mounting). No person who has any regard for his reputation as a microscopist would undertake to diagnose for certain bacilli of tubercle from other similar forms existing in water, urine, or any other medium whatever, whether with a magnification of 200 or 2000 diameters. The property of taking certain aniline stains, and retaining them so firmly that even nitric acid, diluted with only three volumes of water or alcohol, will not bleach them, is one peculiar to the tubercle bacillus, and shared, as far as we know, by the bacillus of leprosy only. This test, along with

* Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 11–13.

† Amer. Mon. Micr. Journ., x. (1889) pp. 277–8; from 'National Druggist.'

isolation and pure culture, alone makes the recognition of bacillus tuberculi certain.

For search of tubercle bacilli and study of the same, we have found a 1/10 homogeneous-immersion objective with a 2-in. eye-piece (approximately 500 diameters) the most satisfactory and least tiring to the eye. A good 1/8, however, with the same eye-piece, should be quite sufficient.

GRANDMAISON, F. DE.—**De l'emploi des solutions de chlorure de zinc pour la fixation des éléments anatomiques.** (On the use of solutions of chloride of zinc for fixing anatomical elements.)
Comptes rend. hebdom. Soc. de Biol., I. (1889) No. 39.

HOYER, H.—**Ueber ein für das Studium der directen Kerntheilung vorzüglich geeignetes Object.** (On an object particularly suitable for the study of direct nuclear division.) *Anat. Anz.*, V. (1890) No. 1, p. 26.

(4) Staining and Injecting.

Practical Notes.*—Mr. H. M. Wilder writes:—*Picric Acid Staining.*—Picrocarmine is very easily washed out with water, at any rate the picric acid. I prefer for that reason to stand the slide on edge, in order to let it drain off, and finally touch the section (or what else) *on the edge* with blotting-paper or filtering-paper, but I do *not* put the blotter *on top*; even the best, and handled most carefully, will always leave fibres. I then allow the section to dry a little, and finally put on the medium. If in balsam I let the section dry thoroughly; the benzole balsam will soon clear it, without any alcohol or oil of cloves. That is for vegetable tissues.

To mount Powders.—In mounting powders I much prefer to breathe on the slide, press it on the dry powder, provided the firmness of the powder is tolerably uniform, give a few smart raps with the edge of the slide on the table, in order to get rid of superfluous powder, put on the cover-glass, with a pencil-brush dust off the surrounding powder, and let the medium *run under* by capillary attraction in the well-known way with a couple of drops on the side of the cover-glass. In this way I seldom have any air-bubbles to contend with.

Silicate of Sodium (soluble glass, water-glass) I would strongly recommend as a medium for vegetable sections and powders. It "sets" quickly, less than fifteen minutes after a mount is made; the slide can be cleaned with a nail-brush without fear of the cover-glass coming off. It clears well, and acts as its own cement, no ringing being necessary. Its disadvantages are: it does not agree with alcohol, ether, volatile oils, mucilage, acids (not even very weak), collodion; being alkaline it will colour lignified tissue yellow, and alter the shades of stains more or less (the bluish-purple colour of hæmatoxylin is turned sepia-brown). After some time it deposits "crystals," that is flakes, which, while they detract from the beauty of the slide, cannot well mislead any one; this tendency may, however, be largely obviated by using a mixture of four or five fluid parts of the silicate and one part of glycerin. This mixture is, of course, slow in drying.

Note.—Mucilage and water-glass do not well mix, because mucilage is always more or less acid; water-glass is very intolerant of acid.

* Micr. Bull. and Sci. News, vii. (1890) p. 17.

Staining of Vegetable Nuclei.*—The following is the method employed by Mr. H. W. T. Wager in staining the nuclei in *Peronospora parasitica*, parasitic on the shepherd's purse (see p. 491). The sections were made by the Cambridge ribbon-section-cutting microtome. The fresh infected tissues of the host-plant were cut up into small pieces, and placed at once either in absolute alcohol or in chromic acid solution, where they were kept until thoroughly penetrated, and were then prepared for imbedding in paraffin-wax. The chromic acid specimens were thoroughly washed in 70 per cent. alcohol, then transferred to methylated alcohol, and finally to absolute alcohol. The pieces of tissue may then be stained *en bloc*, or the separate sections may be stained, when cut, on the slide. The latter gave the best results.

After being thoroughly dehydrated by alcohol, the pieces of tissue were transferred to turpentine for about forty-eight hours, and were then placed in soft melted paraffin-wax for about twenty-four hours, and finally transferred into hard melted paraffin-wax for about two days. They were then imbedded in small square blocks of paraffin, and very thin sections cut by the microtome. These sections were cemented to the slide by a solution of white of egg and glycerin, and the paraffin-wax melted by heating the slide on a water-bath, and washed off in turpentine. The slide was next placed in absolute alcohol, and afterwards transferred to a dilute solution of Kleinenberg's hæmatoxylin in water, made by adding a few drops of the strong hæmatoxylin solution to a beaker of water, until the whole was decidedly coloured. The sections were left in this until they were considerably over-stained, and were then placed in a dilute solution of acid alcohol, made by adding a few drops of strong hydrochloric acid to a beaker of 70 per cent. alcohol for a short time to reduce the stain. They were then washed successively in 70 per cent., 90 per cent., and 100 per cent. alcohol, and were next transferred for a few minutes to turpentine until quite clear and transparent, and were finally mounted in Canada balsam. The preparations thus obtained, which were in many cases only about 1/8000 in. in thickness, exhibited the structure of the nucleus clearly and distinctly.

Nessler's Ammonia Test as a Micro-chemical Reagent for Tannin.†—Mr. S. Moore writes: In most cases the presence of tannin is immediately shown by all the ordinary reagents used by the botanist for its discovery. This does not happen sometimes, however, as, for instance, in the tannin-cells found in the epidermis on the dorsal side of the leaves of some plants. As a good typical example the common primrose may be cited. Of all the ordinary tests, including iron salts, potassium bichromate, Möll's test (copper acetate and iron acetate), ammonium molybdate, and osmic acid in 1 per cent. solution, the latter alone acts immediately upon the tannin in the primrose leaf's epidermis. It may hence be worth while recording the discovery of a second reagent capable of acting rapidly and effectively; and one which is easily made and will keep for some time should be especially valuable. Such a reagent is Nessler's test for ammonia.

Nessler's test is made, as all the world knows, by saturating a solution of potassium iodide with mercuric iodide, and adding an excess

* Ann. of Bot., iv. (1890) p. 131. † Nature, xli. (1890) pp. 585-6.

of caustic potash. Ammonia gives with this a reddish precipitate; tannin a brown, and when in considerable quantity a deep black one; but if little tannin be present, the brown may tend towards purple. It goes without saying that much experiment must be undertaken before one can be sure of the substance giving the brown precipitate being really tannin. To be conclusive, such experiment should be carried out in four different directions:—

(1) The reaction ought to be given in all cases when the ordinary reagents make their presence immediately felt.

(2) Cells which will not immediately give the tannin reaction with ordinary tests, but which will do so with Nessler's test, must also do so under the former conditions if time be allowed.

(3) Tissues, which will not yield the reaction with Nessler's test, must not give it with any other reagent, even after the lapse of some time.

(4) Solutions of tannin must give a brown precipitate with Nessler's test.

Under the first of these headings may be mentioned growing shoots of the garden rose. On laying a radial longitudinal or a tangential section of this in Nessler's fluid, a copious black-brown precipitate is obtained, and the same thing occurs with the beautiful tannin-sacs of *Musa sapientium*. In all other instances, where tannin has betrayed its presence by the use of ordinary reagents, the brown colour has been obtained upon treatment with Nessler's test.

The primrose leaf may be again cited as an example of the time sometimes necessary to show up tannin with the usual reagents, of which it must here suffice to particularize ammonium molybdate. On laying in the molybdate a small piece of epidermis torn off the lower side of the leaf, one first sees a cell here and there coloured the characteristic and beautiful yellow given by this test: these coloured cells are usually situated among the elongated more or less rectangular cells overlying the vascular bundles. Re-examination after half an hour or so shows several more of the cells similarly coloured, but it is usually not till after a couple of hours that one can safely declare all the tannin-containing cells to have been stained. With variations in respect of time, and with the sole exception of osmic acid, all the other tests act in precisely the same way; even Möll's, preferred to all others by some of our Continental *confrères*, being as unsatisfactory as the rest. But sooner or later its characteristic colour is imparted to these cells by every reagent, thus proving tannin to be present.

For the negative experiment the absence of the brown colour from tissues treated with Nessler's fluid, and its absence from the same tissues when acted upon by ordinary tannin reagents, recourse was again had to epidermis. The experiment succeeded in all cases; among these may be cited *Fatsia japonica*, wallflower, box, *Stellaria media*, and *Pelargonium zonale*. In none of these did tannin show up, although twenty-four hours were allowed to elapse before the preparations were destroyed.

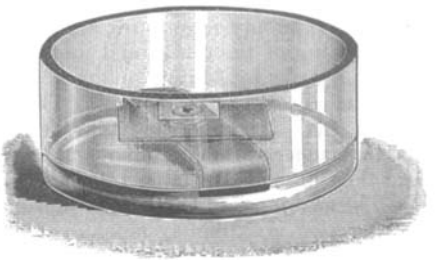
Lastly, Nessler's fluid gives a rich brown precipitate with solutions of tannin. Moreover, with gallic acid a grey-green one is thrown down, thus affording an easy means of distinguishing between these bodies.

For these reasons, therefore, viz. the rapidity, certainty, and distinctness of its action; the ease with which it can be made; its permanence when made; and lastly, the difference in its behaviour towards tannin and towards gallic acid—for these reasons I am bold enough to anticipate the time when, to adapt a hackneyed expression, Nessler's fluid will be regarded as a reagent which no botanical laboratory should be without.

Staining and Imbedding very Minute Objects.*—The preparation of microscopically small objects is usually a very unsatisfactory procedure, but very good results may be obtained, says Dr. E. Overton, by adopting the following method:—Suppose the material is a hanging-drop cultivation on a cover-glass, as for example unicellular algæ, Flagellata, pollen-tube, or the like. When the cultivation has reached the desired stage of development, the cover-glass is removed and iodine vapour allowed to stream over it. Iodine vapour is easily obtained by putting some crystals in a test-tube and warming them. Instead of iodine, osmic acid or its vapour may be used, but then manipulation is extremely difficult, not to say unsatisfactory.

By this method the objects are fixed at once, and then the iodine is removed by heating the preparation up to about 40° for 2–3 minutes. It is sometimes necessary to add a drop of distilled water during the evaporation of the iodine. The cover-glass, with the moist side still uppermost, is then put on a piece of elder-pith, about 3 mm. thick, and with a diameter rather less than the cover-glass. This, in its turn, rests upon a slide (Giessen size), which is placed in a glass capsule, the sides of which are about 2 cm. high. The slide does not lie on the bottom of the capsule, but is placed on a sort of little stool made of metal (see fig. 59).

FIG. 59.



To the preparation is added a drop of 20 per cent. alcohol and absolute alcohol in the capsule, the layer reaching half-way up the stool. The capsule is covered over and sealed up with vaselin. The vessel must be kept at an equal and moderate temperature, and not exposed to the sunlight. In a few hours the alcohol will have acted sufficiently upon the preparation. It is then removed and covered with a drop of collodion, or a solution of celloidin. When the celloidin has set a little, it is immersed in 80 per cent. spirit, wherein it becomes firmly set in about two minutes, so that the preparation may now be placed in any staining solution without fear of damage. The celloidin solution must be quite thin; the author uses the commercial solution diluted with six to ten parts of a mixture of equal parts of alcohol and ether.

* Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 13–16 (1 fig.).

The best stains are carmine and hæmatoxylin, or eosin, iodine-green, and fuchsin. Other anilin dyes, as gentian-violet, are not suitable. The preparations should be dehydrated in 80 to 85 per cent. spirit, and then cleared up with creosote, or with a mixture of equal parts of 90 per cent. alcohol and creosote. They are then mounted in balsam, after having first passed through xylol.

Although this method may appear complicated, in reality it saves a great deal of time.

Surface Deposits in Golgi's Method.*—Sig. P. Samassa, in criticizing Sehrwald's method for preventing surface deposits in sections treated by Golgi's method,† points out that in the original method of Golgi these surface deposits are considerably less. Hence, as in the latter no cover-glass is used, it is an obvious inference that the pressure of the glass sets up diffusion currents, whereby the precipitate is scattered over the section, and renders it often quite useless. The diffusion process is aided by the evaporation of the solvent. In the uncovered method, owing to the large area exposed to evaporation, these diffusion currents are not so likely to occur with such violence as when confined between two rigid layers.

Staining Elastic Fibres and the Corneous Layer of Skin.‡—Herr A. Köppen, in a continuation of the technique of staining elastic fibres,§ recommends a double staining, which may be either diffuse or nuclear.

For diffuse staining the following solution is used:—Carmin optim. 1·0 is dissolved in 50 ccm. cold water, then 5 ccm. liq. ammon. caust. is added, and the whole allowed to stand for two days. It is then filtered, and of the filtrate 1 drop is used to 20 ccm. water. The sections remain therein for twenty-four hours, and are then stained a diffuse red.

Staining of the nuclei and protoplasm.—(1) Weigert's picrocarmine stain is made by adding to the above solution 50 ccm. of a saturated aqueous solution of picric acid. This solution, which should be filtered before and after use, stains in from two minutes to several hours. (2) Grenacher's alum-carmine is made by boiling together for 15 minutes, and then filtering, carmine 1·0; alum 5·0; water 50·0.

The advantage of using these preliminary stains is that the subsequent decolorizing is extremely rapid.

Decolorizing Preparations over-blackened by Osmic Acid.‖—The method of decolorizing objects over-blackened by osmic acid by means of peroxide of hydrogen was, says Dr. E. Overton, first introduced by Fol, but is so little practised that it merits a word in its favour. The following solution, which should be prepared every time, is recommended by the author:—Commercial peroxide of hydrogen 1 part; alcohol (70–80 per cent.) 10–25 parts. The removal of the osmium is completed in a few minutes, and the preparations stain excellently.

Staining Sections of Botanical Preparations.¶—Dr. A. Zimmermann gives a short description of some methods for staining botanical pre-

* Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 26–8.
† See this Journal, *ante*, p. 410.
‡ Zeitschr. f. Wiss. Mikr., vii. (1890) pp. 22–5.
§ See this Journal, *ante*, p. 410.
‖ Zeitschr. f. Wiss. Mikr,, vii. (1890) pp. 10–12.
¶ T. c., pp. 1–8 (1 fig.).

parations, which he has found useful in the examination of chromatophores, crystalloids, and various cytoplasmic elements.

(1) Picro-fuchsin stain.—The sections are fixed to the slide, and the paraffin and its solvent xylol having been removed, are placed in a solution of acid-fuchsin, which is made by dissolving 20 grm. of the pigment in 100 ccm. of anilin water. In this solution, which should be gently warmed, the sections remain for 2–5 minutes, and are then washed in a mixture of 1 part of a saturated alcoholic solution of picric acid and 2 parts of water until no more dye is given off. After this the picric acid is to be extracted in absolute alcohol; then the sections are passed through xylol and mounted in xylol balsam.

(2) Acid-fuchsin staining, with subsequent washing out in flowing water.—This method is serviceable for staining thick sections made from living tissue and then fixed. After the fixative is extracted the sections are placed in a 0·2 per cent. watery solution of acid-fuchsin, in which they remain for 24 hours or longer. The excess of stain is then extracted in flowing water, and this is best done by means of Steinach's glass filter capsules.* The capsules are placed in a receiver, over which is a pipe with a number of small taps, from which the water can be made to flow into the capsules. In adopting this method it is advisable to manipulate a large number of sections at once, and to examine them from time to time to ascertain the proper degree of decoloration.

(3) Iodine-green for staining chromatophores.—The sections are made from tissue previously fixed with an alcoholic sublimate solution, and then immersed for half an hour in a saturated aqueous solution of iodine-green. They are then washed in water and examined in glycerin or Hoyer's mounting fluid, or in balsam. If balsam be used, then dehydration must be effected by merely drying the preparation. Then xylol is added, and, when saturated with this, the xylol balsam. As a contrast stain for the rest of the tissue, a watery solution of Bismarck brown may be used.

(4) Ammonia-fuchsin for staining the chromatophores.—This stain is prepared by adding chemically pure ammonia to an alcoholic solution of fuchsin until the fluid assumes a bright yellow colour. The solution may be used at once, but will only keep a few weeks. The sections are fixed to a slide and some of the solution poured thereon and allowed to remain for some few minutes. They are then washed and examined in water or glycerin. Hoyer's mounting medium may be used, or even balsam. If the latter, then the sections must be dehydrated by drying them in the air.

Staining Human Retina with Acid Hæmatoxylin.†—Dr. J. Schaffer has been able to differentiate the outer and inner segments of the rod and cone layer of human retina by staining the tissue with the acid logwood recommended by Kultschitzky.

The sections, imbedded in celloidin, are taken from the Müller's fluid or alcohol in which they have been fixed and hardened, and left during the night in a 1 per cent. solution of chromic acid, which acts as

* This Journal, 1888, p. 850.

† SB. K.K. Akad. Wiss. Wien, xcix. (1890) pp. 110-20 (1 pl.).

a mordant. After having been washed they are placed in the logwood solution for about twenty hours. The overstaining is removed by decolorizing with Weigert's borax and ferrocyanide of potash solution. The proper degree of differentiation is attained when the rod and cone layer alone remains of a dark colour, the rest of the layers having a brownish hue to the naked eye. The sections are then washed in water and mounted in balsam in the usual manner.

Hæmatoxylin as a means for ascertaining the Alkalinity or Acidity of Tissues.*—Prof. F. Sanfelice has found that the acid or alkaline reaction of tissues may be recognized by staining with Boehmer's hæmatoxylin (alkaline), or with the author's iodized hæmatoxylin (acid).†

In using this method as a test, two principal precautions must be observed. First it is necessary that the normal reaction of the tissue must not be interfered with, hence reagents such as chromic acid and its salts, Müller's fluid and Flemming's solution are unsuitable fixatives. The author used chiefly absolute alcohol for hardening and fixing, and also corrosive sublimate, the excess of which must always be carefully extracted with spirit. The second precaution is that the hæmatoxylin solution must have only a feeble reaction.

Among the instances of differential staining obtained by this method it is mentioned by the author that the protoplasm masses in the ovary and testicle of Selachians are coloured red when the whole of the tissue is treated with the alkaline solution—a fact which proves that the elements undergoing this form of necrobiosis acquire an acid reaction. Goblet-cells in the intestinal mucosa become coloured blue, while the rest of the tissue remains red. Hence the reaction of goblet-cells is alkaline, and this method might be usefully employed to ascertain the reaction of tissues or elements, and their products.

New Method of Staining Central Nervous System, and its Results.‡—Prof. P. Flechsig recommends the following method for staining the nerve-cells of the cerebral cortex and their prolongations. By means of it it was shown that the axis-cylinder process was the only prolongation from the cell which was in connection with a nerve-fibre; that the axis-process, which is not at its commencement medullated, divides like a T, i. e. dichotomously at a right angle. In the occipital lobe a trichotomous subdivision was the rule, although frequent subdivision was also remarked. In the neighbourhood of the central fissure some axis-fibres did not subdivide.

These results were obtained by hardening pieces in 2 per cent. aqueous solution of chromate of potash, and then making sections not exceeding 5/100 mm. in thickness.

After soaking in 96 per cent. spirit, the sections are kept for 3–8 days in a solution of redwood extract at a temperature of 35° C. The sections having been washed in distilled water are then decolorized in the following manner:—Each section is placed in 3 ccm. 1/4–1/5 per cent. solution of permanganate of potash until the solution have lost its

* Journ. de Micrographie, xiv. (1890) pp. 21–2.

† See this Journal, 1889, p. 837.

‡ Berichte ü. d. Verhandl. K. Sächs. Gesell. Wiss. Leipzig, 1890, pp. 328–30 (1 pl.).

bluish colour; it is then immersed in the decolorizer (distilled water 200, oxalic acid 1, hyposulphite of potash 1), until all traces of yellowness have departed from the section.

The redwood solution is made as follows:—1 gram of the pure extract of Japan redwood is dissolved in 10 grams of absolute alcohol, and then diluted with 900 grams of distilled water. To this are added 5 grams of a saturated solution of Glauber's salt and a similar quantity of a saturated solution of tartaric acid.

If this redwood method be combined with Golgi's sublimate staining, the sections, having been stained as above, are placed in a mixture of 20 ccm. absolute alcohol and 5 drops of 1 per cent. solution of chloride of gold and potash, until the sublimate precipitate have become quite black, and the red nerve-fibres have assumed a bluish tone. They are then washed in 10 grams of distilled water, to which 1 drop of a 5 per cent. solution of cyanide of potash has been added, then dehydrated in absolute alcohol, cleared up in oil of lavender, and mounted in balsam.

BURCHARDT, E.—**Eine neue Amyloïdfärbung.** (A new amyloid stain.)
Virchow's Arch., CXVII. (1889).
Cf. *Fortschr. d. Med.*, VII. (1889) No. 23, p. 901; *Centralbl. f. Klin. Med.*, XI. (1890) No. 4, p. 74.

DEKHUYZEN, M. C.—**Ueber das Imprägniren lebender Gewebe mit Silbernitrat.** (On the impregnation of living tissues with silver nitrate.)
Anat. Anz., IV. (1889) No. 25, p. 789.

NICKEL, E.—**Die Farbenreactionen der Kohlenstoffverbindungen. Für chemische, physiologische, mikrochemische, botanische, medicinische und pharmakologische Untersuchungen.** (The colour-reactions of carbon-compounds. For chemical, physiological, micro-chemical, botanical, medical, and pharmacological investigations.) 2nd ed., Berlin (Peters), 1890, 8vo, 134 pp.

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

Finishing Balsam Mounts.*—Mr. F. N. Pease finishes balsam mounts as follows:—The object is mounted on the slide, applying the cover-glass in the ordinary manner, using either balsam, hardened balsam, balsam and benzol, storax or dammar. The slide is then heated to drive off the solvent or more volatile constituents, either gently in a water-bath or at a higher heat, even boiling carefully over a spirit-lamp when the nature of the object will permit. When cold, the superfluous mounting medium is carefully removed, then a narrow ring of paraffin-wax is heated in a capsule until it is melted and quite limpid. With the aid of a very small camel's hair pencil, the melted paraffin is applied at the edge of the cover-glass, covering the exposed medium and instantly solidifying. It is now necessary to apply a finishing cement. For this purpose Bell's cement has been found excellent. If this cement does not work satisfactorily the admixture of some chloroform makes it work smoothly. This cement ring is finished at one application, and in a few hours the slide is ready for the cabinet.

This method is intended to protect the mounting medium from becoming discoloured owing to atmospheric influences.

A new Diatom Mounting Medium.†—Mr. F. W. Weir writes, "C_{10} H_7 Br + Resin of Tolu.—Dissolve 3 oz. of commercial balsam tolu

* Amer. Mon. Micr. Journ., xi. (1890) pp. 66–7.
† Micr. Bull. and Sci. News, vii. (1890) pp. 23–4

in 4 fluid drachms of benzine (C_6H_6) at a temperature of about 45° C., and strain. Add 4 fluid oz. of carbon bisulphide, agitate thoroughly, and allow to cool, when the tolu solution will separate and the carbon bisulphide with cinnamic acid in solution can be decanted. Add another portion of the carbon bisulphide and treat as before. Finally pour the tolu solution into a glass tray and evaporate the benzine.

Place in a 1/2 oz. glass-stoppered phial 1 fluid drachm of naphthaline monobromide, and add gradually about three times its volume of the resin of tolu, or sufficient to make the mixture quite stiff when cold. The solution will be effected slowly at about 45° C. The above constitutes a mounting medium which is rather easier to use than Canada balsam.

Warm the medium at 40° to 45° C. until quite fluid, take up a minute quantity on a warm needle, place on centre of cover-glass and invert on slide. Use no pressure whatever, but warm the slide gently, when the medium will flow to edge of cover.

After a few days ring with a non-alcoholic cement. This method of treating balsam tolu does not remove an atom of resin, and does not allow an atom of cinnamic acid to remain.

The subsequent solution in naphthaline monobromide produces a medium of higher index (1·73) than the resin alone, permanent in structure and volume, and free from objections to which any medium in a volatile solvent is subject."

Tolu and Monobromide.*—Mr. H. L. Smith writes to the Editor of the 'Microscopical Bulletin':—

"I meant to reply to your letter before. The bromide medium will keep if *tightly sealed*, but almost all cements, and some coloured waxes, decompose it. I must say I am not satisfied, and would not advise any one to use it. The yellow medium can be made to keep, but I don't like the colour.

Mr. Weir, of Norwich, Conn., sent me a compound of monobromide of naphthaline and tolu, which is best of any of the high mediums yet—no crystals, easy to use, and very satisfactory.

He is about publishing the formula. I wish somebody—you or some one—would make it for sale, as he does not intend to do this. It has full as high index as monobromide, and none of its disadvantages.

It has consistency of ordinary balsam, and is used like that. It can be hardened by careful heat; or better, mount without heat, and in a day or so it will harden to allow asphalting, or in a few more days will need no ring. It is going to do the thing, I *guess*.

Nothing could please me more than to have you make the bromide medium if I could advise it. It keeps perfectly well in the bottle. I have it two and three years old. No decomposition at all, but it acts so powerfully on all cements, that *this* prevents its usefulness. The index is considerably above monobromide, but the latter is high enough, and I am pretty well pleased with it."

Fixing Sections with Uncoagulated Albumen.†—Dr. J. Rabinovicz has found that albumen may be used for fixing sections to the slide by

* Micr. Bull. and Sci. News, vii. (1890) p. 24.
† Zeitschr. f. Wiss. Mikr., vii. (1890) p. 29.

adhesion as well as by coagulation, and the method is as follows:—The sections are laid on the slide, covered with albumen, and pressed down with a brush. The slide is then put straight into toluol until the paraffin is dissolved. The time required for this varies with the quantity of paraffin (from one to five minutes). The specimen may then be mounted in balsam. If there be any glycerin, however little, mixed with the albumen, this must be removed by immersion of the slide in absolute alcohol for five to ten minutes.

This method has the advantage over others in that it is shorter, and that the albumen is not coagulated by heat or spirit.

(6) Miscellaneous.

New Reaction for Albuminoids.*—Herr C. Reichl proposes the following test for albuminoids, which, though not so sensitive as Millon's reagent, may yet be of service in micro-chemico-botanical investigations. Two or three drops of a dilute alcoholic solution of benzaldehyd, a moderate quantity of dilute sulphuric acid (equal parts of acid and of water), and a drop of solution of ferric sulphate, give a dark blue colour with an albuminoid. A light blue colour is brought out by the first two substances, which becomes deep blue by the action of the ferric sulphate. Concentrated hydrochloric acid may be used in place of the sulphuric, and a different soluble iron salt, for example the chloride, in place of the sulphate.

WHEATCROFT, W. G.—**Presidential Address to the Bath Microscopical Society.** *Journ. of Micr.*, III. (1890) pp. 48–52.

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* SB. K.K. Akad. Wiss. Wien, Monatsheft f. Chemie, 1889, p. 317. See Bot. Centralbl., xlii. (1890) p. 367.