

JOURNAL  
OF THE  
ROYAL  
MICROSCOPICAL SOCIETY;

CONTAINING ITS TRANSACTIONS AND PROCEEDINGS,

AND A SUMMARY OF CURRENT RESEARCHES RELATING TO

ZOOLOGY AND BOTANY

(principally Invertebrata and Cryptogamia),

MICROSCOPY, &c.

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FELLOWS OF THE SOCIETY.

FOR THE YEAR

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## MICROSCOPY.

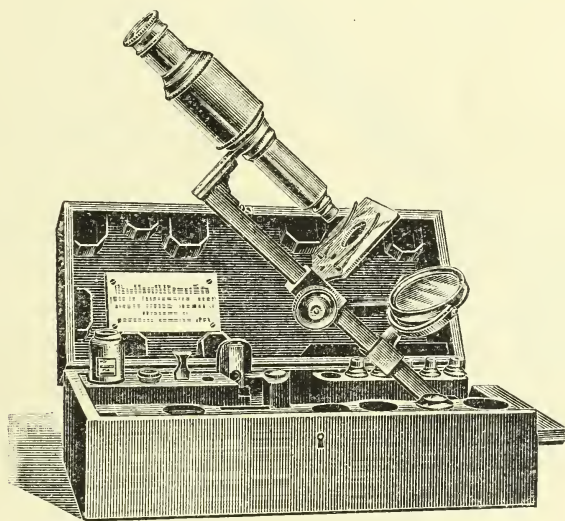
## A. Instruments, Accessories, &amp;c.\*

## (1) Stands.

Sir David Brewster's Microscope.†—At the meeting of the Society, held on November 17, 1897, the President made the following remarks on a Microscope exhibited by Mr. C. L. Curties, which had belonged to Sir David Brewster (fig. 1).

He said that it was used by Sir David Brewster prior to the year 1838. The owner, Mrs. Brewster Ferguson, had presented it to the British Museum, and before sending it there, had kindly sent it through Mr. C. L. Curties for exhibition to the Fellows of the Royal Microscopical Society.

FIG. 1.



At that time Sir David Brewster endeavoured to reduce spherical aberration by constructing lenses of media possessing higher refractive indices than glass, by which means a flatter curve was obtained for any lens of given focus, and for this purpose garnet, sapphire, and diamond were used.

The President said that the Fellows would have an opportunity of judging for themselves the quality of the original garnet lens, as it was exhibited in the room.

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

† There is no maker's name on the instrument; it is similar in construction, and also in its peculiar fitting in its box-foot, to those made by Dollond. Therefore Dollond may with reason be presumed to be its maker.

He had very carefully examined this instrument, and had made measurements of all its parts and calculated its focal distances, and thought it would be interesting to give these particulars. Like many of these older Microscopes, it was fixed to the box, but to the inside and not upon the outside as was usually the case. It could not be taken out of the box, as it was fixed to the inside with a compass joint. Size of box, 12·8 in. long by 5·5 in. wide, by 5 in. deep.

The total length of the body from eye-cap to nose-piece is  $7\frac{1}{2}$  in., and from nose-piece to field-lens 5·3 in.

The stage measures  $2\frac{1}{4}$  by  $3\frac{1}{4}$  in., and is fitted with rackwork by means of which it is adjusted to focus, and there are spring clips provided for holding the slide.

The concave mirror is 2 in. in diameter, with a focus of  $3\frac{1}{4}$  in.; no plane mirror is provided.

The pillar is in length  $9\frac{3}{4}$  in., and in section  $\frac{1}{2}$  in. square; the limb is fixed, the centre projecting 1·8 in. beyond the pillar.

The eye-piece has a doublet compound eye-lens formed of (1) a bi-convex lens with ratio of radii (probably) 1 : 2, the flatter side being toward the eye, diameter 0·8 in., focus 1·3; (2) placed close to the first, an equiconvex lens, diameter 0·9 in., focus 1·5 in.; (3) a field-lens placed at a distance of 1·5 in. from the inner eye-lens; this is also equiconvex, diameter 1·5 in., focus 2·7 in. The joint focus of the two eye-lenses being 0·9 in., and of the whole eye-piece 1·25 in., giving it a magnifying power of  $\times 8$ .

The objectives he found to be as follows:—

No. 1, equal to about a 1 in., power  $\times 50$ —achromatism bad—shows coarse areolations of *Triceratium favus*, and ribs in main cut suctorial pipe in blow-fly's tongue.

No. 2, equal to about a  $1/2$  in., power  $\times 85$ —achromatism good—will not show *T. favus*.

No. 3, about a  $1/3$ , power  $\times 120$ , resolves coarse structure of *Eupodiscus Argus* and ribs in main cut suctorial pipe of blow-fly's tongue; achromatism not so good as that of No. 2.

No. 4, about a  $1/7$ , is the garnet lens, power  $\times 280$ , resolves primary structures in *T. favus*, definition very bad.

No. 5, about a  $1/9$ , power  $\times 360$ , lens equiconvex, achromatism fairly good, resolves all the bars and small hairs in blow-fly's tongue, and primary structure of *Coscinodiscus*.

No. 6, about a  $1/10$ , the best of the series, a doublet, plano over equiconvex, power  $\times 400$ , achromatism fairly good, resolves *Cymbella gastroides*, *Actinocyclus Ralfsii*, and all details of blow-fly's tongue.

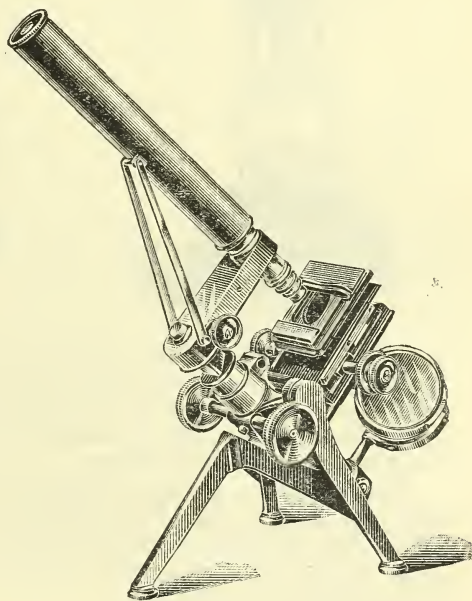
The low power has a large opening, and is consequently very fluffy, but there is a minute hole only at the back of the higher powers, by which the aperture is cut down and rendered exceedingly small. The resolving power is therefore, in the case of the very best lens of the series, only about equal to a  $1\frac{1}{4}$  in. objective at the present day.

**Two Old Microscopes.**—At the meeting of the Society held on December 15, 1897, the President drew attention to two old Microscopes exhibited by Mr. C. L. Curties. The first of these was a Hartnack model, non-inclinable, push-tube coarse, and direct acting screw fine adjustments, plain stage with small round hole, concave mirror, and

metal plate-foot. This instrument had an archaic look on account of the plate-foot, but it was possible to fix an approximate date by the names "Hartnack and Prazmowski" which were engraved upon the tube, Mr. Prazmowski having joined the firm of Hartnack in 1862. There was also another point about this Microscope which helped to fix its date, and that was the Society's screw, this screw having been introduced on November 12, 1857. With regard to the horse-shoe foot, this was adopted by Oberhäuser at the instigation of Mr. Abraham, a Microscope-maker in Liverpool in 1847. This Microscope showed therefore a reversion to the old type.

The next Microscope (fig. 2) possessed a rare form of Powell and Lealand's fine adjustment. It would be remembered that the first adjustment introduced by Mr. Powell was a stage adjustment; a Turrell stage

FIG. 2.



was raised by means of three inclined planes below it, which were moved by a fine screw with a divided head like that of a micrometer, each division equalling the  $1/6000$  in. The date of this was 1833. The Society purchased one of these Microscopes in 1841, and this instrument was upon the table before the meeting. Mr. Powell then, in 1841, altered the entire form of his Microscope, by copying the Jackson model, and by adding a very perfect form of fine adjustment consisting of a cone advancing by means of a fine screw. It was this kind of adjustment, the slide on the Jackson limb, which was so largely employed to-day in the Microscopes of Messrs. Baker, Beck, Swift, and Watson. Mr. Powell's next idea was to adopt the bar movement, the fine adjustment being a long lever of the first order as used at present, but it was interesting to note that the side screw was retained, the Microscope now exhibited being an example of this rare form. This Microscope was dated 1846. Very soon after this the fine adjustment screw was placed in the vertical position it still occupied. He believed that the screw was first placed in the vertical position on the portable Microscope, and he thought it would be found so figured in the first edition of Quekett in 1848. The bayonet-jointed cap to the eye-piece was also a feature of this instrument.

The President also called attention to an interesting old Culpeper and Scarlet Microscope (fig. 3), which differed slightly from those in the Society's collection, three of which were on the table for comparison. The



octagonal form of Marshall's box foot was retained, but the legs were slightly scrolled, instead of being straight, as in the form figured by Smith in his 'Optics,' 1738. Jones, who made a Culpeper Microscope in 1797, placed it on a square box foot, and scrolled the legs more highly, probably for the purpose of giving more room for manipulating the stage

FIG. 3.

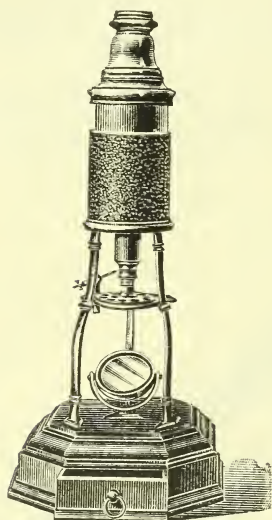
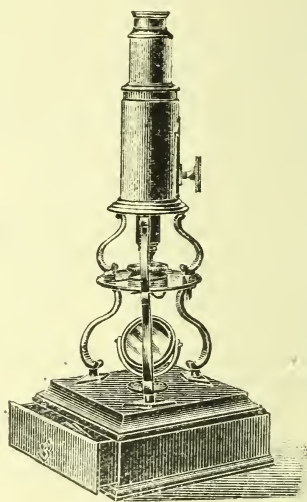


FIG. 4.



and the mirror. In the most elaborate form of this kind of Microscope the body is made of brass, and is fitted with rack-and-pinion coarse adjustment, a fine example of which was lately presented to the Society by Mr. More (fig. 4). The instrument now before the meeting was a very early example with scrolled legs.

**How to Make a Microscope Stand.\***—Mr. W. E. Field gives instructions, illustrated by numerous working drawings, for the construction of a high-class stand; the various operations, from the making of patterns to the finishing of the instrument, being described, especially the details of the metalwork for each part.

### (3) Illuminating and other Apparatus.

**Repsold's New Self-Registering Micrometer.†**—A. Kowalski describes the results of readings taken by an application of this device to an astronomical transit instrument. The principle of the micrometer is that a clockwork arrangement releases or arrests a thread movable over the fixed micrometer threads; and its great value is the almost complete elimination of the correction due to personal equation. The author gives an account of his experience, which seems decidedly favourable.

\* English Mechanic, lxi. (1897) pp. 171, 193, 217, 239, 263, 287 (31 figs.).

† Bull. Acad. Imp. Sci. St. Pétersbourg, May 1897.

## (4) Photomicrography.

**Monochromatic Light for Photomicrography.\***—Mr. A. D. Pretzl enumerates the chief light-filters thus :—Zettnow's chromate of copper, ammonio-sulphate of copper, Fehling's solution, and potassium bichromate, besides signal green, chromium green, and other coloured glasses ; although the light they allow to pass is by no means monochromatic, but practically the whole of the spectrum with certain portions dampened down. In order to make the action of screens thoroughly understood, the following tables of reference are necessary :—

Fraunhofer Lines.	Wave- Length. $\lambda$	Fraunhofer Lines.	Wave- Length. $\lambda$
A . . . . .	759	E . . . . .	527
B . . . . .	687	F . . . . .	486
C . . . . .	656	G . . . . .	430
D . . . . .	589	H . . . . .	397

## DISTRIBUTION OF COLOURS (AFTER LISTING).

	$\lambda$		$\lambda$
Red . . . . .	723-647	Bright blue . . . . .	491-455
Orange . . . . .	647-586	Blue violet . . . . .	455-424
Yellow . . . . .	586-534	Violet . . . . .	424-397
Green . . . . .	534-491		

Zettnow's cupro-chromate filter allows light from wave-lengths 570-550 to pass, and is made by dissolving 160 grains of pure dry cupric nitrate and 14 grains of chromic acid in 250 c.cm. of water. The thickness of liquid should be 1 cm. The ammonio-sulphate of copper allows light from 475-400 wave-lengths to pass, and this is prepared by dissolving finely powdered sulphate of copper in four times its weight of liquid ammonia (sp. gr. 0.96). When diluted or with a thin stratum of solution, light of 515  $\lambda$  will get through.

Fehling's solution cuts off the extreme red and the ultra-violet. The potassium bichromate absorbs the violet, blue, and bluish-green, according to its strength and thickness.

Landolt's filters, as used for polariscopic work, should be equally good for microscopical work, and the following directions for making them may be useful.

*Red filter.*—Crystal violet, 5 BO, 0.05 grm. should be dissolved in a little alcohol and diluted to 1000 c.cm. with water, in a trough 20 mm. thick ; this gives a red band with a broad blue violet band, which can be absorbed by a 10 per cent. solution of potassium chromate. The red stripe left begins about  $\lambda$  718 and ends abruptly at  $\lambda$  639.

*Yellow filter.*—Dissolve 30 grm. of nickel sulphate in 100 c.cm. of water, and in a thickness of 20 mm. this absorbs the red only ; a 10 per cent. solution of potassium chromate in 15 mm. thickness absorbs the blue, and a 15 mm. thickness of 0.025 grm. of potassium permanganate in 100 c.cm. of water absorbs the green, leaving  $\lambda$  614 to  $\lambda$  574.

*Green filter.*—Cupric chloride ( $\text{CuCl}_2, 2\text{H}_2\text{O}$ ), 60 grm. dissolved in 100 c.cm. water, and 20 mm. thickness, passes the green and blue, and a

\* English Mechanic, Dec. 1897, pp. 358-9.

10 per cent. solution of potassium chromate in 20 mm. thickness absorbs the blue, leaving  $\lambda$  540 to  $\lambda$  505.

*Bright blue filter.*—Doppelgrün, SF 0.02 grm. dissolved in 100 ccm. of water and in 20 mm. thickness, leaves a narrow red band, a broad green band with blue; and 15 grm. of cupric sulphate dissolved in 100 ccm. of water in 20 mm. thickness, absorbs the red and green, leaving  $\lambda$  526 to  $\lambda$  458.

*Dark blue filter.*—0.005 grm. of crystal violet 5BO dissolved in 100 ccm. of water in 20 mm. thickness, and the sulphate of copper solution as used above, also in 20 mm. thickness, allow  $\lambda$  478 to  $\lambda$  410 to pass.

### B. Technique.\*

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

**Plankton Gathering.**†—Mr. G. Murray recommends, for use with deep-sea plankton, a cylindrical silk bag, about  $1\frac{1}{2}$  ft. long and 3 or 4 in. wide. This was tied to the nozzle of the hose, there being a lateral overflow vent near the top of the bag. On pumping through this with the donkey engine from an intake pipe 8 ft. below the surface, good results were obtained. This contrivance enables the operator to work while steaming, and is often convenient when the weather is too rough for tow-netting. The fixative and preservative employed was a 0.5 per cent. solution of chromic acid; good results were also obtained with Fleming's solution, and with platinic chloride of various strengths.

**Culture of Diatoms.**‡—Dr. H. van Heurck publishes a *resumé* of M. Miquel's admirable methods for the artificial cultivation of diatoms. The cultures are divided into ordinary and pure, and subdivided into cultures of fresh-water and salt-water diatoms.

(1) Ordinary cultures of fresh-water diatoms are grown in a fluid containing salts and organic substances. The saline nutriment is prepared in two solutions:—A. Sulphate of magnesium 10 grm., chloride of calcium 10 grm., sulphate of sodium 5 grm., nitrate of ammonium 1 grm., nitrate of potassium 2 grm., nitrate of sodium 2 grm., bromide of potassium 0.2 grm., iodine 0.1 grm., water 100 grm. B. Phosphate of sodium 4 grm., chloride of calcium sic. 4 grm., hydrochloric acid 2 ccm., perchloride of iron liquid at 45° 2 grm., water 80 grm. These solutions are mixed when required for use, in the proportion of 40 drops of A and 20 drops of B to 1 litre of tap-water, and then are added 5 cgrm. of wheat straw and a similar quantity of earth moss, previously washed in boiling water. Small cultures may be made in wide-necked flasks plugged with cotton-wool; large cultures in crystallisers, or in the square jars formerly used as electric accumulators. It is useful to sterilise the fluids in a water-bath for a quarter of an hour at 70°. The medium is inoculated with living frustules. The cultures should be kept from the sun and exposed to a northern light at 10°–30° C. The growth of green algæ may be diminished by reducing the light.

\* 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.

† Journ. of Bot., xxxv. (1897) pp. 387–8.

‡ Zeitschr. f. angew. Mikr., iii. (1897) pp. 193–8, 225–36.



Every 10 or 15 days the water lost by evaporation should be replaced by sterilised water, and if the cultures drag, their growth may often be stimulated by adding a few drops of solutions A and B.

(2) Culture of marine diatoms is easily done in sea-water. If, however, natural sea water cannot be obtained, it may be replaced by dissolving sea-salt 250 grm., sulphate of magnesium 20 grm., chloride of magnesium 40 grm., in a litre of water, and when required for use adding 9 litres more. To the sea-water are added the solutions A and B, just as for fresh-water diatoms, and also a little *Zostera* leaf.

(3) Pure cultures of diatoms are obtained by the fractional method, or by isolating a single healthy specimen. The former procedure is effected by mixing one drop of diatomiferous fluid with 100 ccm. of nutritive fluid, and diluting 1 ccm. thereof with 99 ccm. of fresh fluid. The last solution is then distributed in ten Freudenreich's flasks and cultivated. In this way cultures of a single form may be finally obtained and further examined in a cell devised by Miquel (see p. 130).

Dr. van Heurck then gives the methods and formulæ used by the late Mr. C. Haughton Gill. The fluid from which Mr. Gill obtained the best results was composed as follows:—Solution A. Chloride of sodium 10 parts, sulphate of sodium 5 parts, nitrate of potassium 2·5 parts, acid phosphate of potassium 2·5 parts, water 100. B. Filtered spring water 100 vols., solution A 0·5 vol. To this solution is added a sufficient quantity of slaked lime to neutralise the acidity of the liquid, and a small quantity of precipitated silica. Finally, a small quantity of sterilised grass infusion or diatom broth, obtained by prolonged boiling of a large quantity of fresh diatoms in water. After filtering the broth, it is preserved in hermetically sealed tubes. Fine bone-scrappings were also added, and occasionally well washed grass roots.

Though the foregoing gave very excellent results, Mr. Gill, in the last year of his life, adopted a fluid more like that of Miquel. This was a mixture of four different solutions:—

Solution i. Pure crystallised phosphate of sodium 2; pure crystallised chloride of calcium 4; pure syrupy perchloride of iron 0·5; pure hydrochloric acid 1; water 100.

Solution ii. Pure crystallised sulphate of magnesium 4; pure crystallised sulphate of sodium 4; pure crystallised nitrate of potassium 4; common salt 8; bromide of potassium 0·2; iodine 0·2; water 100.

Solution iii. Pure crystallised carbonate of sodium 4; water 100.

Solution iv. Silicate of calcium, precipitated and washed, 25; water 75.

Of each of these solutions 3 ccm. were taken and mixed with 1 litre of tap or sea water, and the mixture distributed in Erlenmayer's flasks of 100–200 ccm. capacity, which were filled up to a height of 3 ccm. The flasks were plugged with cotton-wool, sterilised, and left for at least one week before being inoculated. Solution iii. has a double action, for it neutralises the acidity of the fluid and precipitates half of the calcium as useful carbonate. Mr. Gill was of opinion that the purity of the substances used was highly important, especially of the perchloride of iron, the ordinary samples often containing arsenic, one of the most fertile causes of failure. Direct sunlight was harmful, and the culture flasks were placed facing W.N.W., a screen of pale-green glass being inter-



posed. The flasks were inoculated with one or more frustules isolated by means of a capillary tube.

**Miquel's Cell for Pure Cultures of Diatoms.\***—Dr. H. van Heurck describes Miquel's cell for the pure cultivation of diatoms. To a slide having a circular hole 2 mm. in diameter towards its upper side is fixed a ring, and to the ring a circular cover-glass. In this way is made a cell with a lateral aperture, which can be held vertically or horizontally. The slide is allowed to rest during the cultivation on the cover-glass upon which the deposits form, and hence these cultures may be examined with the highest powers.

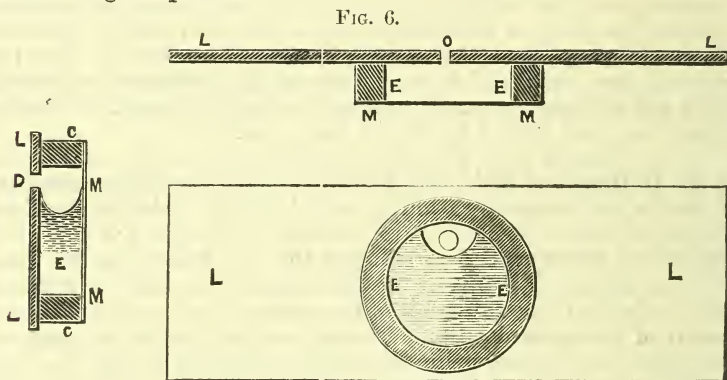


FIG. 5.

FIG. 7.

Fig. 5.—Transverse section of cell: L L, slide; D, aperture of 2 mm.; c c, ring; M M, cover-glass; E, cultivation fluid.

Fig. 6.—Longitudinal section: O, aperture.

Fig. 7.—View of cell from Microscope bent at an angle.

**Cultivating *Gonococcus*.†**—Dr. A. Wassermann has found that *Gonococcus* grows best on a medium which contains uncoagulated serum-albumen and pepton. The coagulation of the serum by boiling is prevented by the addition of casein-sodium phosphate (nutrose), a substance which also promotes the growth of *Gonococcus*. The medium is made by putting 15 ccm. of pig's serum in an Erlenmeyer's flask, diluting with 30-35 ccm. of water, and then adding 2-3 ccm. of glycerin, and finally 0.8 grm. (2 per cent.) of nutrose. The flask is then well shaken in order to properly mix the ingredients, after which it is heated to boiling. It is sterilised in 20-30 minutes. The sterilised solution may be kept in this condition for future use, and suffices for 6-8 plates. When required for use, a number of 2 per cent. pepton-agar tubes are liquefied and mixed in equal parts with the serum fluid, and the mixture poured into Petri's capsules. When set, the medium is ready for use. The serum and agar must be mixed at between 50° and 60°. If the pig's serum is very rich in albumen, it is advisable to mix the 15 ccm. with 40 ccm. of water instead of 35 ccm.

\* Zeitschr. f. angew. Mikr., iii. (1897) p. 230.

† Berlin. Klin. Wochenschr., 1897, p. 685. See Centralbl. Bakt. u. Par., 1<sup>te</sup> Abt., xxii. (1897) pp. 486-7.

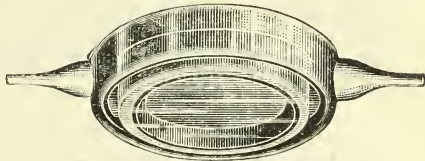
**Nutrient Gelatin with High Melting-point.\***—The three chief points to be carefully attended to in obtaining a gelatin medium with high melting-point are, says Prof. J. Forster, the height of the temperature acting on the gelatin, the duration of the action, and the concentration of the gelatin. In practice, this amounts to observing that the temperature should never exceed  $100^{\circ}$ , and should not be protracted longer than 40 minutes, and that the gelatin should amount to 5 per cent.; for it was found by experiments conducted for the purpose that the melting-point of gelatin was lowered by protracted boiling, and by the greater amount of water in the medium. It was further determined that above 5 or 6 per cent. the melting-point was but little higher than at these amounts; consequently on the whole it was more advantageous to compose the medium with 5 per cent. On these lines a nutrient gelatin is produced which, after standing for 24 hours, has a melting-point between  $29^{\circ}$  and  $30^{\circ}$  C.

**Capsule for Anaerobic Cultivation.†**—Dr. M. Beck has devised a modification of Petri's capsule which renders this apparatus useful as a moist chamber, and for the cultivation of anaerobic organisms. By giving an S-bend to the flange of the cover, two furrows or grooves are

FIG. 8.



FIG. 9.



produced, the upper one of which serves for the reception of water when the capsule is to be used as a moist chamber. By the addition of a couple of lateral tubes any gas can be introduced. When required for anaerobic cultures, the lower furrow is filled with paraffin and fixed to the edge of the capsule by mere pressure. In this way an air-tight chamber is produced.

**Seminal Fluid as a Nutritive Medium.‡**—Dr. A. Cantani, jun., obtained pure seminal fluid by removing the testicle and spermatic cord and squeezing out the fluid on to the surface of oblique agar. As the fluid was removed aseptically, sterilisation and antiseptic precautions were avoided.

Testicular juice which was smeared over the surface of oblique agar was obtained by cutting through the testicle, and removing the juice with a platinum loop. The tubes were afterwards incubated for 10 hours.

Though most bacteria grew fairly well on the seminal media, only influenza bacilli gave really good results.

**Influence of the Reaction of the Medium on Bacterial Growth.§**—Herr M. Deeleman has made experiments for the purpose of ascer-

\* Centralbl. Bakt. u. Par., 1<sup>o</sup> Abt., xxii. (1897) pp. 341-3.

† Tom. cit., pp. 343-5 (2 figs.).

‡ Tom. cit., pp. 601-4.

§ Arb. a. d. K. Gesundheitsamt, xiii. (1897) pt. 3. See Centralbl. Bakt. u. Par., 1<sup>o</sup> Abt., xxii. (1897) p. 355.

taining whether the proportion of alkali advocated by Maassen and used in the Imperial Laboratory at Berlin is the correct one, viz. 1·035 ccm. per cent. normal soda solution to 1 litre litmus-blue neutral bouillon, and also whether the addition of caustic soda or of soda is more advantageous for bacterial growth. Twenty different species of bacteria were used for the research. The conclusions the author arrived at were:—

(1) The addition of soda for the great majority of the bacteria examined was found to be more advantageous than caustic soda. In a small number of bacteria there was no difference between the two. With diphtheria the growth was usually better with caustic soda than with soda. With anthrax the growth was invariably better with caustic soda.

(2) A slight addition of alkali beyond the litmus-blue neutral point was advantageous for most bacteria, only *B. pyocyaneus* and *B. cyanogenus* thriving better on neutral media.

(3) The optimum lay between 0·34–1·7 ccm. per cent. normal caustic soda solution, and 0·39–1·95 ccm. per cent. normal soda solution. The addition of 1·15 per cent. of crystallised soda over the litmus-blue neutral point, corresponding to 1·05 ccm. per cent. normal caustic soda solution, as used by Maassen, seems therefore suitable for most bacteria.

(4) The limits for good growth lay generally between 1·7–3·4 ccm. per cent. normal caustic soda solution and 1·95–3·9 per cent. normal soda solution. With diphtheria, however, the limits reached were only 1·0 ccm. per cent. and 1·17 ccm. per cent.; while with *B. ruber Plymouth*, *B. erythrogenes*, *V. Miller* they were 5·1 ccm. per cent. and 5·85 ccm. per cent. respectively.

#### (2) Preparing Objects.

**Apparatus for Filtering Bacterial and other Fluids.\***—Dr. F. G. Novy describes a filtering apparatus, the principal advantage of which is that the positive pressure from the compressed air co-operates with the negative pressure of a pump in filtering the fluid, so that in 3–5 minutes 250 c.cm. of water can be passed through.

The essential parts of the apparatus are a glass cylinder, and a Chamberland bougie, which is inserted within the cylinder. The latter has a flange at the lower end, by means of which, through the intermediation of clamps and caoutchouc rings, it is firmly fixed to the bougie.

**Simple Steam Steriliser.†**—Dr. F. G. Novy recommends a simple apparatus for steam sterilising. The lower part is the ordinary Hoffmann's iron water-bath, 18–20 cm. in diameter. The upper part, made of copper, has a perforated bottom. Inside are soldered two rings 1½ cm. broad, of perforated copper, one about 4 cm., the other about 12 cm. from the bottom. The object of these rings is to prevent the culture tubes from touching the sides. The lid is furnished with a tube for the escape of the steam.

#### (4) Staining and Injecting.

**Staining Yeast-Cells.‡**—Herr O. Busse, in his work on yeasts as causes of disease, recommends the following method for staining these

\* Centralbl. Bakt. u. Par., 1<sup>o</sup> Abt., xxii. (1897) pp. 337–9 (2 figs.).

† Tom. cit., p. 340 (1 fig.).

‡ 'Die Hefen als Krankheitserreger,' Berlin, 1897. See Centralbl. Bakt. u. Par., 1<sup>o</sup> Abt., xxii. (1897) p. 349.



organisms. Stain for 15 minutes in alum-hæmatoxylin, wash in water, and then stain with very dilute phenol fuchsin (1 part Ziehl's solution with about 20 parts of distilled water) for 30 minutes to 24 hours. After this, decolorise and dehydrate for 15 seconds to 1 minute in alcohol, then 95 per cent. spirit followed by absolute alcohol, xylol, and balsam.

**Method of Treating Bacteria difficult of Staining.\***—M. L. Dubois adopted the following procedure when dealing with bacteria difficult of staining. To 10 ccm. of a 25 per cent. solution of tannin, a solution of ferrous sulphate was added until the liquid was of a black colour. The mixture was heated to 50°, and the cover-glass films immersed therein for 25 minutes. The cover-glass was next transferred without being washed to a 1 per cent. solution of potash. After 15 minutes the film was washed, and finally hot-stained with anilin-oil fuchsin solution, and the preparation examined in water.

**Rapid Staining of Tuberculous Sputum.†**—Dr. N. P. Andrejew recommends the following method for staining tuberculous sputum. The cover-glass films are first stained in the usual way with the phenol-fuchsin solution, and are then treated with the following mixture:—(1) hot 10 per cent. potassium chlorate solution, 100 ccm.; (2) acid green (Grübler) 1 gm.; (3) 25 per cent. pure sulphuric acid 15 ccm. The mixture, after having been well shaken and filtered, forms a dark green fluid in which the stained cover-glass is immersed until the film becomes of a green or bluish-green hue (about 1 minute). After this the cover-glass is thoroughly washed with tap-water, and then dried on blotting-paper. It may be examined at once, or mounted in the usual way.

The chief advantages claimed by the author for this method are that, as green is the complementary colour to red, the red stained objects are better seen, and also that only two solutions are required.

**Intra-Vitam Staining.‡**—Herr A. M. Przesmycki has made a long series of experiments on *intra-vitam* staining, and has improved the methods. His general results are thus summed up:—Different organisms react differently, the various organs differ in their receptivity, even in the same animal; different parts of the same organs may be differentiated; the parts coloured *intra vitam* lose their colour after death, or when the organism is placed in fresh water without any pigment; the method makes it possible to show certain details which are not demonstrable in any other way.

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

**Preserving Sea-Anemones.§**—N. Kholodkovsky recommends douching sea-anemones (in a little sea-water) with 40 per cent. formol diluted ten times with fresh water. The animals die uncontracted, and the colours remain for at least two weeks, when the author—somewhat prematurely perhaps—communicated his recipe.

\* Comptes Rendus, cxv. (1897) p. 791.

† Centralbl. Bakt. u. Par., 1<sup>o</sup> Abt., xxii. (1897) pp. 593-7.

‡ Biol. Centralbl., xvii. (1897) p. 353.

§ Bull. Soc. Zool. France, xxii. (1897) p. 161.



**Flemming's Fixing-Solution.\***—Herr D. M. Mottier recommends the following composition for studies in nuclear division in plants:—

1 per cent. chromic acid .. ..	16 ccm.
2 per cent. osmic acid .. ..	3 ccm.
Glacial acetic acid .. ..	1 ccm.

**Preservative Fluids for Botanical Specimens.†**—M. J. Chalon recommends a saturated or 3 per cent. solution of boric acid for preserving vegetable specimens. This was the only one out of ten different solutions which gave satisfactory results. The solution is improved if 1 to 5 per cent. sodium sulphate be added.

The other solutions tried were, 2 per cent. calcium chloride; 0.25 per cent. chromic acid; chromo-acetic acid; Müller's fluid; salicylic acid; carbolic acid; corrosive sublimate thymol; sodium sulphite. The use of formalin in 3 per cent. solution is alluded to, but its advantages or disadvantages are not mentioned.

#### (6) Miscellaneous.

**New Method of Observing Stomates.‡**—Prof. F. Darwin thus describes a new method used by him for observing the opening and closing of stomates:—"A strip of horn sheet 8-9 mm. long by 3-4 mm. wide is fastened by one end to a small block of cork so that the horn lies flat on any surface on which the instrument is placed; the horn bearing at its free end a bristle to serve as index. When it is placed on a damp surface the index instantly rises to an angle of 20-50°, or even more, whereas it remains flat on a dry surface. A paper scale being fixed to the cork block serves to read off the movement; when applied to leaves, e.g. a hypostomatal leaf, the reading is zero on the upper side, and varies on the under side according to the state of the stomates; 50° means an extreme amount of variation, 30° a fair degree, 10° a small degree."

**Detection of Typhoid Bacilli in Fæces by Elsner's Method.§**—Dr. S. Sterling writes in praise of Elsner's method for the detection of typhoid bacilli in the fæces. As previously stated, the essential feature consists of potato-gelatin with 1 per cent. iodide of potassium, which medium is found to be unsuitable for most of the microbes from fæces, and has the further merit of distinctly differentiating the colonies of *B. coli* from those of typhoid, those of the former being yellowish and granular and of fast growth, while those of the latter are small transparent droplets. The author states that his percentage of positive results with Elsner's method is 66, while other procedures did not give a higher percentage than 16.5.

**Method of rapidly Identifying the Microbe of Bubonic Plague.||**—

The method devised by Mr. E. H. Hankin and Surgeon-Captain B. H. F. Leumann for rapidly identifying the plague bacillus is derived

\* Jahrb. f. wiss. Bot. (Pfeffer u. Strasburger), xxx. (1897) p. 170.

† Bull. Soc. Roy. Bot. de Belge, xxxvi. (1897) pp. 39-46.

‡ Proc. Cambridge Phil. Soc., ix. (1897) pp. 353-8.

§ Centralbl. Bakt. u. Par., 1<sup>te</sup> Abt., xxii. (1897) pp. 334-6. Cf. this Journal, 1896, p. 357.

|| Tom. cit., pp. 439-40.

from the observation that in old agar cultures large and peculiar involution forms are common; and in that about to be described those involution forms may under favourable conditions be obtained within 24 hours, and thus become a means for rapidly identifying the plague microbe. The microbe is to be inoculated in agar containing 2.5-3.5 per cent. of salt, and incubated at 37°. In 24, and certainly within 48 hours, every single bacillus will be swollen up and altered so that they resemble spheres, spindle-shaped, or oval bodies, and occasionally torulæ, appearances which cannot easily be mistaken for any other known microbe. In the case of certain bacilli having a superficial resemblance to those of plague, isolated involution forms recalling those of plague may be met with, but it will never occur that the whole culture is so changed.

In carrying out the test it appears to be necessary to first cultivate the microbe on ordinary agar and then transfer it to salt agar. The authors have also found that potassium bromide or iodide can be used in the place of sodium chloride in a strength of about 2 per cent., but this variation of the method does not seem to present any special advantage.

**Microscopic Study of Alloys.\***—The following notice of M. Charpy's important work on this subject appears in the issue of 'Nature' for November 4, 1897, with the signature "T. K. R."

"The study of metals with the Microscope proceeds apace, and is now becoming as generally pursued among metallurgists as the determination of melting-points has been during the last five years. Since the appearance of Prof. Roberts-Austen's article on 'Micrographic Analysis' of iron and steel, a large amount of work has been done; but most observers still devote themselves more or less exclusively to the study of this metal, attacking unsolved problems which seem to have great industrial importance. This tendency is unfortunate from some points of view; for the complex constitution met with in that protean element makes it less easy to explain the observed appearances until, by work on simpler alloys, a better acquaintance with the whole subject has been obtained. M. Charpy is one of those who has resisted the temptation offered by the alloys of industry, and in a recent paper has given some interesting results of his investigations on binary alloys which are well worth re-statement.

"It is now fairly established that microscopic examination gives an immediate analysis of alloys, which is all the more valuable for differing in its results from chemical analysis, since these differences indicate the existence of definite compounds, and elucidate the structure in other ways. The immediate analysis is now made with the aid of a planimeter, as Sauveur recommended, by which the ratio of the areas occupied in the microscopic field by the various constituents can be measured. The metal or metals forming each of these constituents can often be indicated by their colour, hardness, and, above all, the effects on them of various reagents, and thus a full account of the alloy can be given.

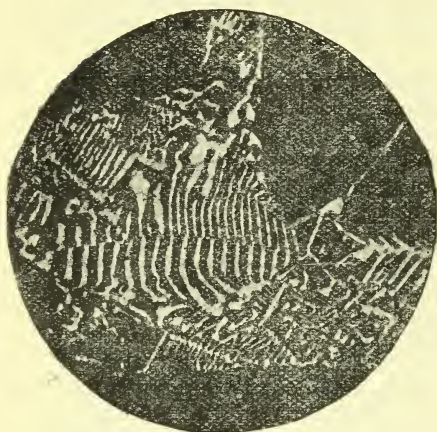
"In the normal type of constitution of binary alloys, crystals of one

\* 'Étude Microscopique des Alliages Métalliques.' Bull. Soc. d'Encouragement, ii. (1897) p. 384.

of the metals, or of a definite compound of the two, are seen enveloped in a second constituent, which is generally the eutectic alloy, containing both elements in a very finely divided state. The composition of the eutectic mixture remains constant, whilst the amount of isolated crystals varies with the percentage composition of the alloy. The limiting cases of a pure definite compound or metal, and of a pure eutectic mixture, may be grouped with these alloys.

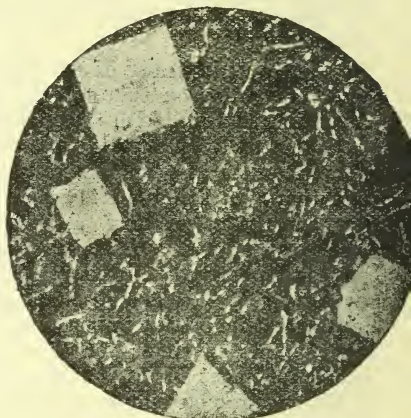
"Eutectic alloys vary in appearance according as they have been cooled slowly or quickly. In the latter case, the surface is uniformly striated, but the crystals or crystallites are so small that it is difficult to obtain satisfactory photographs of them. When the solidification is slow, however, the separation into lamellæ is strongly marked, especially when viewed under high powers, and this structure is highly characteristic of eutectic alloys, being easily traced in any of them whatever the

FIG. 10.



Alloy of silver, 66 per cent.; antimony,  
34 per cent.

FIG. 11.



Alloy of tin, 90 per cent.; antimony,  
10 per cent.

metals in the alloy may be. It is well shown in fig. 10, which represents an alloy containing silver 66 per cent., antimony 34 per cent., magnified 500 times; the metal has been treated with sulphuretted hydrogen, which has blackened the silver and left the antimony unchanged. In the same figure some straight edges can be seen, in which the ramifications end, and which sketch out shapes resembling those of crystals of antimony. The presence of these crystallites or incipient crystals in eutectics constitutes one of the resemblances between them and the micro-felsitic basis observed in many igneous rocks, and it seems likely enough that if light transmitted through these alloys could be examined, it would show that they are on the borderland between crystalline and amorphous matter.

"Besides the normal type of binary alloys, in which eutectics are observable, there is a second type consisting of alloys of metals which form

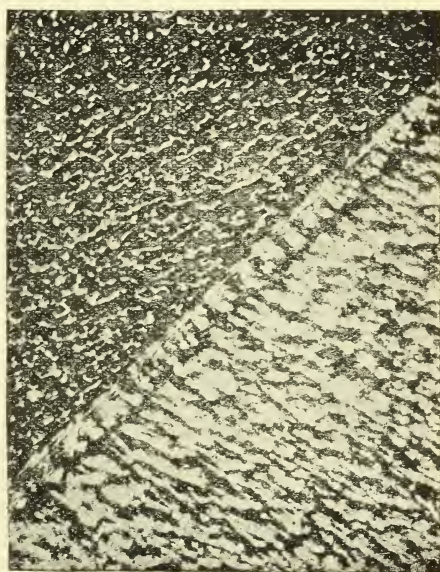


isomorphous mixtures with each other. These alloys, whatever may be their composition, consist of only one species of crystals, which fill the whole space, the composition and the properties of the alloys usually varying in a continuous manner in each crystal. The number of metals capable of forming isomorphous mixtures with each other is small, the bismuth-antimony alloys being the only ones out of fourteen series investigated by M. Charpy in which this property was found to exist; but, on the other hand, there are many cases of definite compounds of two metals isomorphous with one of them. Thus, for example, microscopic study has enabled M. Charpy to detect a compound of tin and antimony containing about 50 per cent. of tin and isomorphous with antimony,

FIG. 12.



FIG. 13.



Alloy of tin, 75 per cent.: antimony, 25 per cent.

Pure gold.  $\times 1000$  diameters.

although the freezing-point curve, worked out by Roland-Gosselin, and consisting of three branches having their concavities upwards, and meeting in two angular points or maxima, gives no direct indication of the relation between these metals.

"In fig. 11, in which the alloy containing 10 per cent. of antimony is shown, the cubical crystals appear to consist of the 50 per cent. alloy set in a eutectic magma. Fig. 12 shows the alloy with 25 per cent. of antimony. As the proportion of antimony in the whole mass approaches 50 per cent., these crystals invade the whole field, and numerous minute cracks appear, on the edges of which is seen a secondary crystallisation without the interposition of an intermediate substance. This structure is characteristic of a pure or homogeneous substance, as in the beautiful micro-sec-



tions of pure gold prepared by Osmond and Roberts-Austen,\* one of which is reproduced in fig. 13. When the proportion of antimony is increased above 50 per cent., a eutectic magma shows no signs of reappearing. Similarly in the tin-antimony series, there is evidence of a compound containing 20 per cent. antimony and isomorphous with silver, and in the silver-tin series a compound containing 30 per cent. of tin also appears to be isomorphous with silver. An investigation of the triple alloys of these metals would be interesting, as probably affording fresh examples of isomorphous series."

\* Osmond and Roberts-Austen, 'On the Structure of Metals, its Origin and Changes,' Phil. Trans., clxxxvii. (1896) A, pp. 417-32.

III.—*The Application of the Electric Arc to  
Photomicrography.*

By J. EDWIN BARNARD, F.R.M.S., and THOS. A. B. CARVER, B.Sc.

(Read 17th November, 1897.)

IN its application to photomicrography the electric arc presents many important advantages. In addition to its inherent simplicity, the high degree of intensity and the smallness of the area of the incandescent source, effect corresponding improvement of the image, and render short exposures practicable. Photographic results which would not be available with other sources of illumination can further be obtained from nearly opaque objects. It has, however, hitherto been employed with small success; and it was with the object of localising the difficulty, and if possible rectifying it, that the investigation to be described was undertaken.

The result of numerous trials of the best form of automatic lamp available showed that, although the arc was working under constant conditions so far as the most careful adjustment could secure, the photographs obtained were subject to wide variation. Not only did the results of equal exposure demonstrate fluctuations in the intensity of the light, but shadows and diffraction phenomena were produced which were only to be attributed to decentration of the illuminant. The difficulty was even accentuated by the fact that at times the most perfect results were obtained; but their reproduction was a matter entirely of chance. Our first experiments were therefore devoted to the analysis of the behaviour of the arc when thus controlled. The method we adopted was to project an image of convenient size upon a screen provided with lines, in reference to which the movement of the image could be referred.

Our observations showed that, although working under apparently constant conditions, the arc was subject to wide fluctuations, on account of the movement of the carbon points due to the feeding mechanism, and of the incandescent point travelling round the carbon as the result of the variations in the length of the arc. In order to test the true effect of these variations, the automatic mechanism was replaced by a simple form of hand-fed apparatus, in which absolute positiveness and steadiness of movement was secured, so that the conditions observed could be reproduced and studied at length.

No further experiment was, however, necessary to show that in the unsteadiness of the feeding motion of the carbons lay part at least of the decentering action to which were to be ascribed the variations we had observed in the photographic result.

With regard to the effect of inconstancy of the length of the arc, our results showed that so long as the carbons were maintained at a constant critical relative distance the intensely incandescent crater, from which practically the whole of the useful light is derived, remained absolutely motionless and of constant intensity, affording from a photomicrographic point of view an almost perfect illuminant. So soon, however, as the slightest lengthening of the arc occurred, the incandescent crater is lost, owing to the arc at once travelling round the positive carbon, playing successively upon different parts, and never remaining long enough in one place to allow the high incandescence and constancy of position secured when running under

FIG. 24.

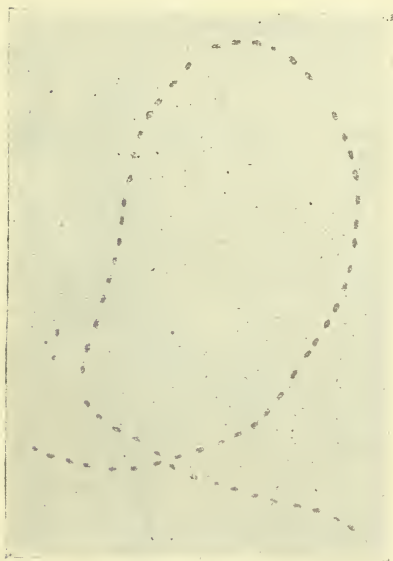


FIG. 25.



the critical condition we have described. In order to more directly test the effect of the small variations we had observed, two photographs were taken: one with the crater of the positive carbon truly central (fig. 24), and the other (fig. 25) with the crater decentered by an amount, indicated by projection, corresponding to the fluctuations of the automatic lamp.

We were thus led to realise the importance of higher accuracy in the adjustment of the position and length of the arc; and to consider means whereby it could be secured. The necessarily intermittent action of any automatic mechanism, the action of which can only be started as the result of the very factor it was our object to avoid, viz. lengthening of the arc, forced us to the conclusion that such

constancy and accuracy of movement could only be attained by such a simple positive hand-fed apparatus as that to which we have alluded. The form we designed to meet the condition is shown in fig. 26.

The carbons are held in V-clamps sliding upon two rigid vertical rods, and their relative positions can be varied by the right and left handed screw A; the position of the pair, that is of the arc as a whole, being controlled by the screw B. The lamp is mounted upon a boss sliding upon a tube, and has movements through the angle of inclination and in a line at right angles to the line of collimation.

FIG. 26.

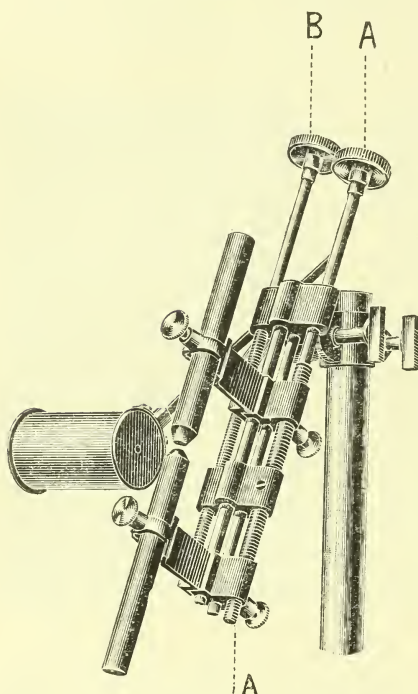
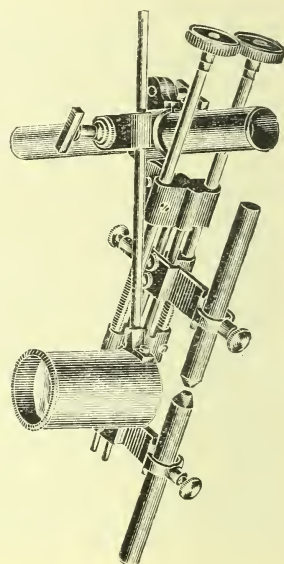


FIG. 27.



With such mechanism the arc was fed to maintain its truly central position and constant condition, in reference to the graduations upon the screen, and any difficulty in its use entirely disappeared, and the reproduction, even with the highest powers, of the best photographic results became a matter of ease.

It remained to design means whereby the indications of the projected image could be observed in a smaller compass, and we were ultimately led to adopt a pin-hole casting an image upon a screen of ground glass provided with the necessary reference lines. Such an



apparatus we designed as part and parcel of the feeding mechanism, providing it with a universal motion and a clamp, so that, the light having been adjusted to be truly central, the indicator can be placed in a position to then read correctly upon the ground glass. In fig. 27 the lamp is shown, with pin-hole camera attached, at right angles to the optical axis.

The question of inclination of the carbons to the vertical was investigated by means of an apparatus giving revolution of the arc about a horizontal axis passing through itself.

The critical inclination is obviously the greatest at which none of the light from the crater is cut off by the negative carbon, when the length of arc has been adjusted to the greatest that gives constancy in the position of the crater.

The critical angle we found to be about  $27^{\circ}$  to the vertical, and at this angle the two essential conditions are secured, viz. the greatest amount of light was thrown forward from the crater of the positive carbon and the light from the negative is thrown to the back, and consequently eliminated as a second and harmful source.

It is of course understood that the current employed throughout these experiments has been a continuous one. It remains to state how ill the alternating current adapts itself to such an application.

None of the conditions which our experiments with continuous current showed to be so primarily essential are present. In the first instance, the arc can never be adjusted to give that constancy of one incandescent source which we were led to place at the root of any success in the use of the arc. Again, the carbons are equally incandescent, so that it is impossible to regard the arc as a single source of illumination. These essential difficulties are further regardless of minor physical inconveniences, such as the noise, which, although they might not form barriers to its use, are nevertheless absent with continuous current.

We venture to think that, could the electric arc be regarded as a reliable source of light, it would be much more largely employed in optical apparatus where constancy of position and intensity of the source of light are essential, and can only express the hope that our results demonstrate the ease with which these conditions can be accurately secured.

IV.—*A new Form of Photomicrographic Camera and Condensing System.*

By E. B. STRINGER, B.A.

*(Read 15th December, 1897.)*

THE apparatus which Mr. Watson Baker has kindly undertaken to submit to the Society on my behalf, is an attempt to facilitate the work of photomicrography, especially with high powers, and to afford an illuminating system more perfectly corrected, more powerful, and more under control, than has hitherto been available.

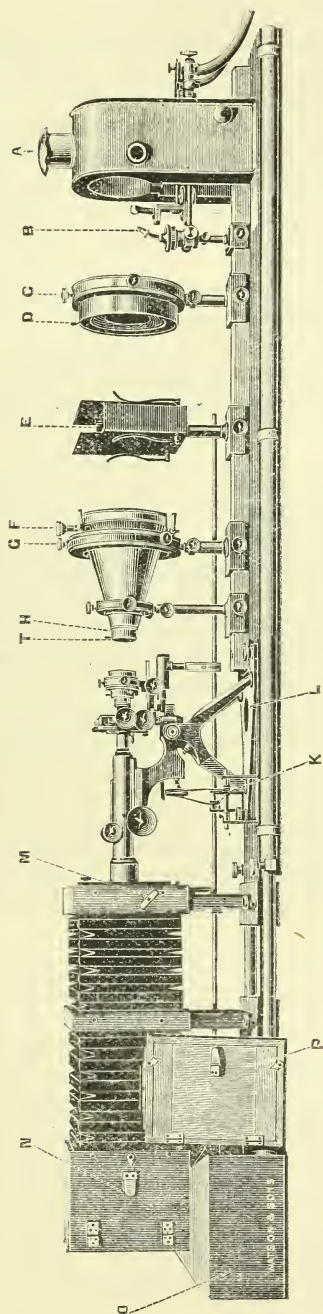
The baseboard is confined to that part of the apparatus which carries the illuminating system and Microscope, the further end of the camera being supported by a massive wooden block. To this are attached two long brass tubes, on which slide the supports for the bellows, and which themselves slide in slightly larger tubes attached to each side of the baseboard. By drawing out these inner tubes with the block, and sliding the bellows supports the other way, the camera can be extended to 40 inches, or it may be closed up to 11 inches; but at whatever length it may be used, there is no baseboard projecting beyond the end of it, and the focusing screen is always in the most convenient and accessible position, namely at the end of the bench, where the worker can be comfortably seated. Also the firm support of the massive wooden block is always immediately beneath it, conferring the greatest possible solidity and freedom from vibration.

A door is provided at the side of the camera for the examination and adjustment of the image *in situ*, when a white card is substituted for the ground glass or other screen.

If it should be necessary, though in practice it very seldom is, to look down the Microscope when it is in position, the camera can readily be drawn backwards altogether out of the way of the observer's head. And it will be seen that the whole camera can in a moment be entirely removed, and the Microscope used, if so desired, for screen projection.

I have found that the secret of avoiding vibration is to clamp down nothing, but to let everything rest by its own weight on as large a surface as possible. To increase this effect, and to bring the apparatus into firm and intimate contact with the bench (and also to help to absorb vibration), it has underneath it a layer of sheet cork one-eighth of an inch thick, below which is another layer of felt also one-eighth of an inch thick; on these it rests with great firmness, and at the same time slides easily enough when pushed.

FIG. 28.



B, Oxhydrogen jet with zirconium cylinder, covered by the cowl A when working.

C, Doublet parallalising condenser, with centering screws.

D, Iris diaphragm.

E, Holder for trough and light-filtering media.

F, Plano-convex lens  $4\frac{1}{4}$  in. diameter, with centering screws G.

H, Plano-concave lens, with iris diaphragm T.

K, Connecting pulleys between focusing rod of camera and fine adjustment of Microscope.

L, Triangular frame in which Microscope feet are placed.

M, Flap shutter.

N, Door through which image is observed on card screen, &c.

O, Solid block of mahogany on which camera body is fixed and supported.

P, Dark slide.



At the end of the baseboard next the camera is a triangular plate of brass, having three holes into which the round feet of the Microscope (Watson's H Edinburgh stand) accurately fit, the feet passing completely through the holes and resting on the board beneath, which is here covered with cloth. At the three angles of the plate are three milled screws, passing through holes which (when the screws are unclamped) are large enough to allow about a quarter of an inch of free movement in each direction. When the Microscope is first put down, these screws are released, and the Microscope shifted until it is found to be exactly centered. The screws are then firmly clamped, and are never again touched. The Microscope can now be instantly put down in an accurately central position into the holes which receive the feet, and as easily taken up again. This enables the Microscope used for ordinary observation to be employed for photography with as much ease and accuracy as the permanently fixed instrument found in some arrangements.

The small model instrument is preferred, not only because it is lighter and more convenient to move about, but also because its optic axis when in the horizontal position is lower; thus the optic axis of the whole apparatus, and consequently its centre of gravity, is lower, and its stability and freedom from tremor the greatest possible. The small light body-tube also confers a greater sensitiveness on the fine adjustment than can be obtained in the full-sized instrument; and it will be shown farther on how the small tube may be used, without any danger of flare from internal reflection.

The "turn out" device is dispensed with as being unnecessary where the Microscope is not fixed down. All adjustments are easily made, with the powerful illumination provided, by projecting the image on to a screen of white card.

The front of the camera has sliding movements in both directions, by which the connecting flange can be once for all adjusted to exactly meet the eye-piece of the Microscope. Behind it is the usual flap shutter for making the exposures, worked by a large milled head outside the camera.

The focusing arrangement adopted is the one in which an endless cord passes over the fine adjustment screw and round two other pulleys below and on each side of it, by which any lateral drag on the Microscope is altogether avoided. These pulleys can be changed, and used of various sizes, those for high power work being as small as it is possible to make them, so that a very slow movement is easily obtained. The focusing rod passes through the block at the end of the camera, and terminates in a large milled head, which, whatever length of camera may be in use, is always close to the focusing screen and the left hand of the worker.

In extending or closing up the camera there is only one screw to be released, namely, the small one which clamps the focusing pulley on to the focusing rod. The clamping screws on the ends of the

lateral tubes and those on the bellows supports are adjusted to a convenient tightness, and are not afterwards touched.

The condensing system, the oxyhydrogen jet, and a small lantern of sheet iron, are all carried on the optical bench, which I have found it a great improvement to make square in section, instead of prismatic as has been usual heretofore. With the condensing system many difficulties were at first experienced, until Mr. E. M. Nelson very kindly interested himself in the matter, and computed a new set of condensers, following the same general plan as I had done, but making them larger ( $4\frac{1}{4}$  inches in diameter) and employing the new Jena glasses. These, after one or two final alterations, proved entirely successful, and the result (the perfection of which is due to Mr. Nelson's optical knowledge and skill) is an illuminating system perfectly achromatised, and almost perfectly aplanatic, and moreover of much greater power than has hitherto been available, affording a beam of great intensity, and of that slight divergence which is best suited to the working of the substage condenser.

The working of the whole system is as follows:—The light from the jet is first parallelised by the doublet condenser, which consists of two plano-convex lenses having their plane sides turned towards the radiant, the one next the radiant being a quarter of an inch less in diameter than the other, and the focal length of the combination being  $2\frac{3}{4}$  inches, taking up an angle of  $70^\circ$ .

The parallel beam passes across an interval of about 10 inches, through the screen or trough of coloured solution carried by the intervening support, and enters the plano-convex lens  $4\frac{1}{4}$  inches in diameter (having its convexity turned the other way to minimise the aberrations), by which it is converted into a converging cone. This, after passing through the water in the chamber between the lenses, is again parallelised by the much smaller plano-concave lens, and emerges from it as a parallel, or more strictly speaking a slightly divergent pencil, rather less than an inch in diameter, and enters the substage condenser. The plano-concave lens is of highly dispersive glass, and perfectly achromatises the whole system.

The converging arrangement is clamped at such a distance from the substage condenser as to throw a spot of light rather larger than the opening in the condenser diaphragm. The smaller the radiant point, or the larger the opening in the diaphragm, the greater this distance should be. The best average distance is about  $2\frac{1}{2}$  inches from the substage ring as it stands at present. This also allows ample room for putting down and taking up the Microscope, and for using the mirror when the Microscope is in place, if it should be necessary to do so. For "dark ground" work, or in using the Lieberkühn, the small iris may be removed, and the nose of the converging system slid right up into the substage ring, so that there is no loss of light whatever.

The small weighted cone regulator, attached either to the main

or to a tube of compressed hydrogen, supplies gas to the "mixed" jet (which has a much smaller nipple than usual) at a pressure of 2 inches of water; so that, with the hydrogen tap full open, the light is always of the same power, and exposures may be timed with certainty. The oxygen is better taken at a higher pressure direct from a tube having only the usual spring regulator, and controlled by the screw tap of the jet; but it is as well that the spring should be a weak one. The zirconia cylinder which is used, with the jet directed on to the end of it, gives an incandescent point almost as small as the electric arc, and is at the same time much more manageable, and in every way superior to lime for the present purpose. With it the beam emerging from the first combination is almost perfectly parallel. If a larger radiant such as a lamp-flame or Welsbach burner be used, it is much better to bring up the back combination as near to the other as the intervening support will allow.

It will be noticed that not only is the emergent beam entirely robbed of its heat by passing through so large a depth of water, but the jet is, in virtue of the whole arrangement, removed to so great a distance from the Microscope, that there can be no indirect communication of heat, such as might affect the focal adjustment in high power work. The illumination is also of such power that very deep and approximately monochromatic screens may be used, with moderate exposures; and excellent work may thus easily be done with objectives which are not apochromatic.

The device of the parallelising plano-concave lens is of course originally due to Kingsley, who first employed it in a Microscope for screen projection about the middle of the present century, and it has since been used by Mr. Lewis Wright in his oxyhydrogen lantern Microscope, now so well known.

The large iris in front of the combination next the light is an important feature. By it the substage condenser is focused and centered, and when it is shut down to the right extent, it will be found (besides rendering the whole system perfectly aplanatic) to entirely cut off that surplus light, which when reflected from the inside of the Microscope tube and objective mount, causes so much trouble and so many failures in photomicrography. So that it is no longer necessary to use a specially large tube, and the small tube, with the greater sensitiveness it confers upon the fine focal adjustment, can be retained. If no light whatever is to strike the sides of the tube (though a little does not of course matter) the diaphragm of the substage condenser must not be opened beyond its full aplanatic aperture.

There is, besides, a small iris beyond the plano-concave parallelising lens which cuts off any remaining stray light, and is also used for centering.

The whole system is provided with centering screws, and it, like the Microscope, is centered once for all and never again touched. The



only adjustment that has to be made with each fresh exposure is the centering of the light itself (which is for this purpose provided with very sensitive centering screws), and the adjustment of its distance from the condensers, which will be found to vary a little when different objectives and substage condensers are used. The small sheet-iron lantern slides in grooves on each side of the optical bench, and, when slid backwards, gives easy access to these screws.

Finally, it must be said, that to employ this illuminating arrangement to the greatest advantage, it is necessary that the substage condenser be proportioned in power as well as in aperture to the objective. The more nearly the power of the substage condenser approaches that of the objective, the wider can the large iris be opened, and the greater is the intensity of the illumination; but it is the intensity alone which is affected; the aperture of the substage condenser, whatever it may happen to be, remaining unaltered. The best results appear to be obtained when the condenser is about one-half the power of the objective.

If this proportion be pretty closely maintained, and the other conditions are fairly equal, as is often the case in the general run of work, the exposure varies but little with varying magnifications, averaging about two seconds with a light yellow screen and a plate of medium rapidity.

The apparatus was made for me by Messrs. Watson in the summer of 1896, and I must, in concluding, express my thanks to them for the care and thoroughness with which they have carried out my design.

## MICROSCOPY.

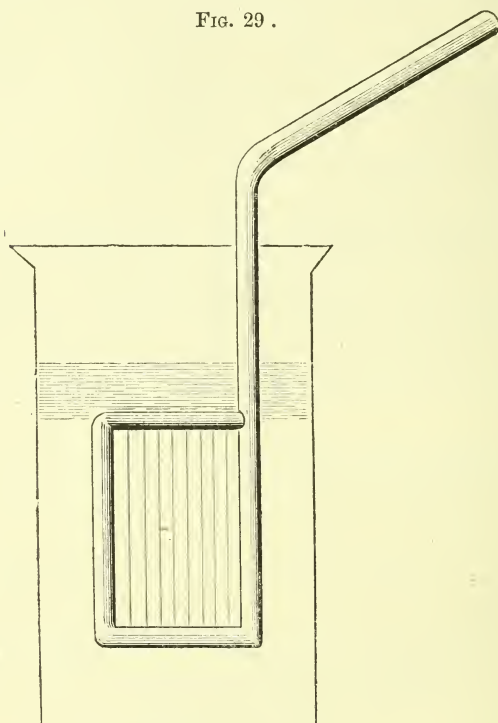
[An abstract of Dr. Clifford Mercer's important paper on "Aperture," referred to in the President's Annual Address, is unavoidably postponed to the next number.—EDITOR, JOURN. R.M.S.]

## A. Instruments, Accessories, &amp;c.\*

## (3) Illuminating and other Apparatus.

**Spider-Lines.**†—Mr. F. L. O. Wadsworth reports very favourably on his experience of quartz-fibres instead of silk or spider threads in the

FIG. 29.



cross lines of optical instruments. The transparency of the quartz-fibres is removed by silvering them by any of the ordinary methods. The difficulties arising from the hygroscopicity and semi-translucency of both

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

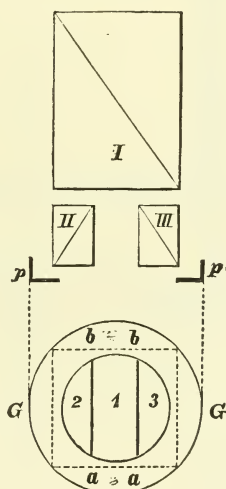
† The Microscope, December 1897, p. 157.

silk and spider threads are thus overcome, and the quartz, while equalling these other materials in fineness, surpasses them in strength. Mr. Wadsworth asserts that anybody, after a little practice, can easily make the fibres, the only essential apparatus being an oxyhydrogen blow-pipe which must be fed by compressed oxygen and fairly pure hydrogen (coal gas is unsatisfactory). He has found no method for "shooting" the fibres superior to the bow and arrow method first used by Prof. Boys. Previous to immersion in the silvering solution, the fibres should be chemically cleaned by successive immersions in (1) strong nitric acid; (2) distilled water; (3) strong caustic potash; (4) distilled water. He finds it convenient to mount a dozen fibres in a glass rod frame, fastening them with fused shellac which resists both hot acid and hot alkali. The frame can then be laid aside and the fibres removed for use when required.

**Novelties in Polarisation Apparatus.\***—W. Wicke classifies saccharimeters into:—(1) Apparatus for circular polarisation requiring under all circumstances homogeneous illumination—usually sodium light; and (2) apparatus with wedge compensation and linear scales which can be used with white light.

(1) This includes the old forms of:—Mitscherlich, Wild, Halb-

FIG. 30.



schatten-Laurent, and Lippich. The best is that of Lippich, which excels in accuracy and certainty; its advantage depending upon a tripartition of the field of view.

(2) This includes Soleil's saccharimeter depending on a now antiquated colour system, with the improvements by Ventzke and by Scheibler, as well as applications of the half shadow (*Halbschatten*) system. Soleil, although still much used, is unsatisfactory, both in

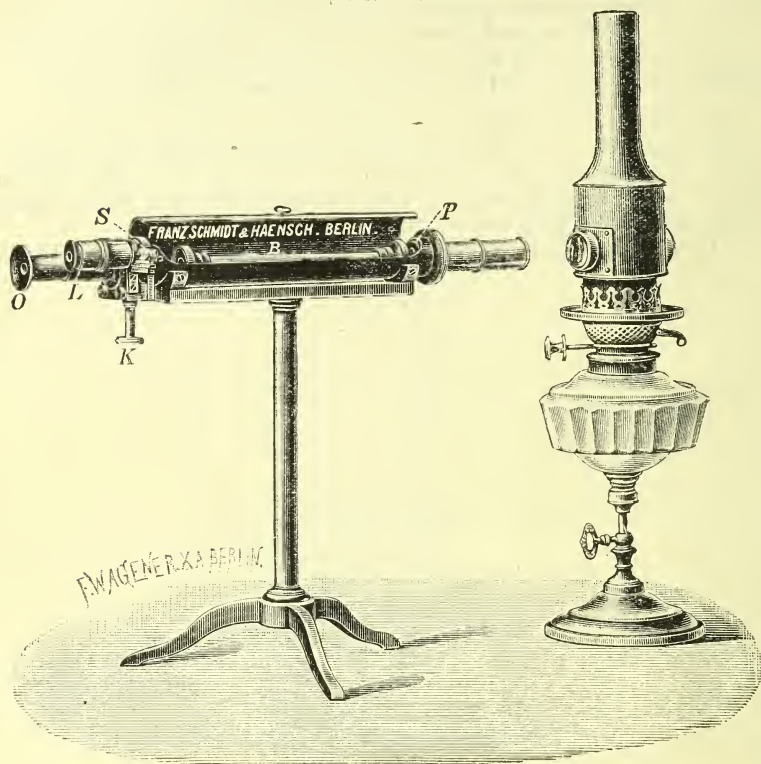
\* Berichte der Deut. Pharm. Gesells., 1898, pp. 7-15 (7 figs.).



cases of colour-blindness and on account of the variation in colour sensation.

Fig. 31 shows the external form of the simple half-shadow apparatus with wedge compensation, as specially constructed for urine analysis; and fig. 32 shows the method of arranging the lenses. The advantages of Laurent's half-shadow are larger dimensions, greater delicacy of reading, and better mechanical arrangement.

FIG. 31.



In Lippich's tripartition of the field of view, a much greater improvement of the half-shadow results, securing a very high degree of accuracy. Whilst in the simple Lippich polariser only one sharp-edged so-called half-prism is placed before the large nicol, and the field of view bisected, in the tripartition system two of these sharp-angled half-prisms are employed, as shown in fig. 30. The effect of this application is to produce as great an improvement in the wedge compensation as in the circular apparatus.

With the Landolt-Lippich apparatus the investigation of substances in strongly heated or cooled media is possible (fig. 33). For such purposes a metal box with asbestos casing and an inner observation tube

gilded inside (fig. 34) are used. Nickel scales take the place of ivory, as they are found not to bulge with changes of temperature. A mirror

FIG. 32.

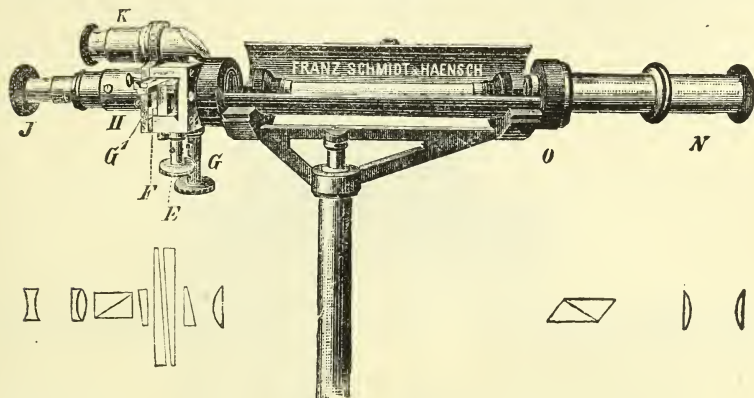
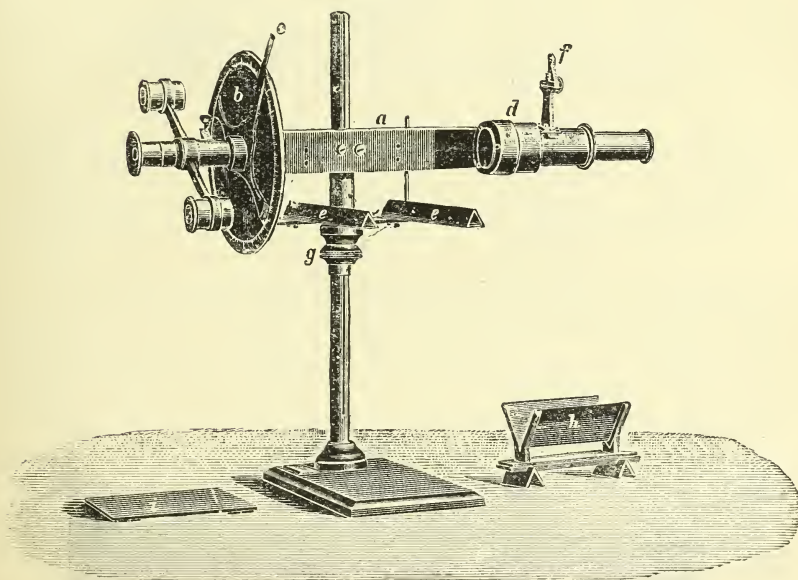


FIG. 33.



S adjusted by a ball-and-socket joint K allows the light from the lamp to be reflected from a mirror L on to the nickel scale B (fig. 35).

The lamp has also been much improved. For circular apparatus a grooved platinum ring has been substituted for the platinum net,

formerly used for the sea-salt; this gives a very bright uniform yellow flame. For white light the improvements include two and threefold

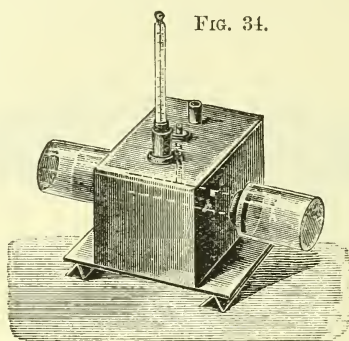


FIG. 34.

flat burners on the cylinder. Electric and incandescent lamps have also been used with good effect.

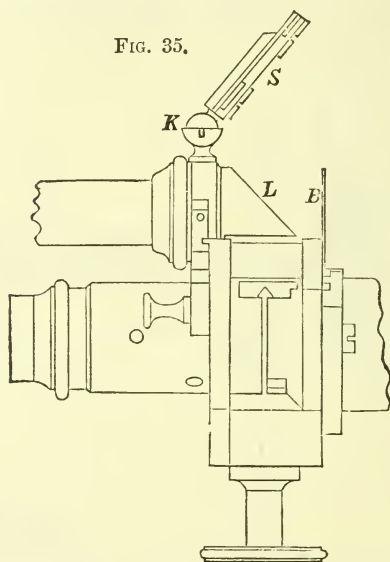


FIG. 35.



FIG. 36.

To avoid optical activity in the liquid, the observation tube is enlarged in its centre and filled so as to permit the enclosure of an air-bubble, which, when the tube is *in situ*, does not interfere with observation (fig. 36).



## (6) Miscellaneous.

**Laboratory Dish.\***—Prof. W. M. L. Coplin describes the dish used in his laboratory in the manipulation of paraffin sections. As the illustration (fig. 37) shows, the vessel is made of glass; it has the following measurements:  $3\frac{1}{2}$  in. high, 1 in. square at bottom,  $1\frac{3}{8}$  in. square three

FIG. 37.

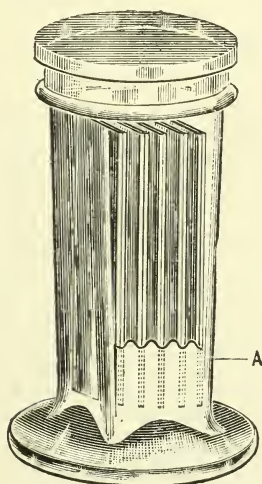
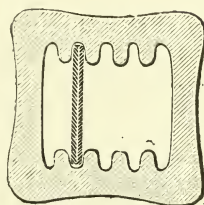


FIG. 38.



inches from bottom,  $1\frac{3}{8}$  in. in diameter at top, which is round and covered with an air-tight cover; the base is nearly  $2\frac{1}{2}$  in. in diameter. The inside is ridged on two opposite sides (fig. 38), so that five grooves are formed in which eight slides may be packed, three pairs back to back and one at each end.

**Micro-Sclerometer for Determining the Hardness of Minerals.†**—Mr. T. A. Jagger, jun. (Camb. Mass.) describes an instrument which appears to give a new and valuable method of determining the hardness of minerals, whether simple, compound, amorphous, or crystalline. The quality which it is proposed to measure is the resistance offered by a body to the removal of particles of its substance by a defined diamond point moving in contact with it under uniform conditions. The instrument is applied to the Microscope, so that it may be used for either thin sections or crystal faces. The adjustments of the instrument are such that any of the variable elements in the process of abrasion may be made functional while the others are maintained constant. The principle is as follows:—A diamond point of constant dimensions is rotated on an oriented mineral section under uniform rate of rotation, and uniform weight to a uniform depth. The number of rotations of the point, a measure of the duration of the abrasion, varies as the resistance of the

\* Journ. New York Mic. Soc., xiii. (1897) pp. 87-9 (2 figs.).

† Amer. Journ. Sci., Dec. 1897, pp. 399-412 (1 pl. and 2 figs.).

mineral to abrasion by diamond: this is the property measured. The instrument (plates III. and IV.) consists of the following parts:—

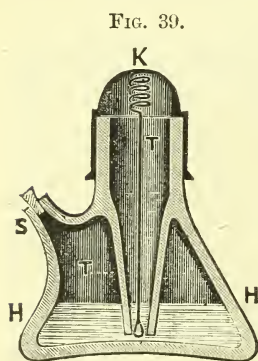
1. A standard and apparatus for adjusting to Microscope.  
(i) foot adjustments; (ii) rotating adjustments; (iii) lifting adjustments; (iv) fixing adjustments.
2. A balance beam and its yoke.
3. A rotary diamond in its end.
4. Apparatus for rotating uniformly.
5. Apparatus for recording rotations.
6. Apparatus for locking and releasing.
7. Apparatus for recording depth.

The instrument admits of measurements with any of the four variables,—rate, weight, depth, duration. The last has been found most practical, because it gives the highest values, and hence admits of the most delicate gradation.

Weights (e.g. 10 grm.) are placed on the pans *w* (plate IV.), and a micrometer scale at *m*; *D* is the diamond, which has to be carefully selected, and has a perfect point. The plate *m* is rotary on a horizontally pivoted ring, so that it may be turned in any azimuth. This device is so adjusted that the micrometer scale is visible in the field of the Microscope at the point exactly 10 mm. from the axis of rotation of the diamond point; this is one-sixth of the distance from the diamond axis to the beam pivots *a*, hence any downward movement at the diamond point is magnified by one-sixth at the micrometer. The reading is therefore seven-sixths of the actual depth bored. If now it be rotated until the micrometer scale stands at right angles to the beam, and be then tipped gently, an inclination may be found where, under a high power, only one line of the micrometer scale is in focus at a time, and a downward focus of precisely .01 mm. is necessary to bring the next lower line on the slope into focus. Conversely, if we focus on the lower line and allow the diamond to bore its way down .01 mm., the next higher line of the micrometer glass will come into sharp focus only when that depth is reached. We thus have here an extremely sensitive measure of depth.

The author gives a preliminary series of tests with the minerals of the Mohs scale to show the efficacy of the method. The improvement of certain details is under consideration. The instrument seems to be applicable to the determination of the amount of double refraction, and to the measurement of the thickness of mineral thin sections.

**Immersion-Oil Bottle.\***—Dr. W. Gebhardt describes a new form of bottle for holding cedar oil for immersion purposes. As the illustration (fig. 39) shows, the bottle is much like a small Erlenmeyer's ask with a side tube *S* and a fairly broad neck *T*, the latter being



\* Zeitschr. f. wiss. Mikr., xiv. (1897) pp. 348-50 (1 fig.). J.

prolonged downwards to near the bottom of the bottle, and being narrowed so as to form an elongated funnel. The neck T is closed by a metal cap K, from inside of which passes a wire ending in a loop. The loop picks up just the necessary quantity of oil, provided that the bottle be not filled too much. Owing to its broad base the bottle is very steady, and if S be closed with a cork there is no fear of any overflow, as the bottle then acts as a siphon. For travelling, the metal cap must be replaced by a cork.

### B. Technique.\*

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

**Alkalised Serum as a Culture Medium for Diphtheria Bacilli.**†—Dr. L. Cobbett prepares alkalised ox-serum in the following way. To every 100 ccm. of the serum 2 grm. of glucose and 1.75 ccm. of a 10 per cent. solution of NaHO are added. The mixture is then put into tubes and sterilised in an autoclave at a higher pressure than usual. This is effected by closing the exit tap of the autoclave before the air which it contains has been expelled, and thus the pressure of the heated air is added to that of the steam. The medium is of a rich brown hue, and should be perfectly transparent by transmitted light. On it the colonies are discrete, flat, grey, or almost colourless, and after several days' growth their margins usually became indented and radially fissured.

Alkalised horse-serum, from which more rapid results are obtained, is prepared as follows. To every 100 ccm. of serum 2 grm. of glucose and from 1.25 to 1.2 ccm. of a 10 per cent. solution of NaHO are added. The mixture is then poured into tubes and Petri's dishes, and sterilised at a temperature of 90° C. on 2 successive days in a chamber surrounded by a jacket containing boiling water. Thus prepared the medium is bright, transparent, and almost as light in colour as gelatin. Colonies may be seen on it after from 6 to 8 hours' incubation; the colonies are, however, not so radially striated (daisy-shaped) as with ox-serum.

The impression method of examining the growth is recommended, as thereby a large number of colonies can be examined at once.

**Preserving Living Pure Cultivations of Water Bacteria.**‡—Mr. J. Lunt finds that water bacteria can be preserved in sterilised tap water for considerable periods without impairment of vitality or of their characteristic and specific features. This simple method has the following advantages. (1) It obviates the necessity of frequently transplanting cultures to fresh media; such cultures remain in good condition for periods during which they would have died on the ordinary media. (2) The cultures are preserved in their natural habitat, and, when re-sown, grow vigorously. (3) The method serves also to differentiate those organisms which may properly be classed together as water bacteria from those outside the water group.

The experiments were made with *B. fluorescens liquefaciens*; *fluores-*

\* 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.

† Lancet, 1898, i. pp. 362-3.

‡ Trans. Brit. Inst. Preventive Med., 1st series, 1897, pp. 152-63.



*cens fuscus* sp. n.; *violaceus*; *nubilus*; *iridescentis*; *prodigosus*; *giganticus* sp. n.; *coli communis*.

**Actinomycotic Form of the Tubercle Bacillus.\***—Herr P. L. Friedrich injected rabbits with 0·2–6·5 ccm. of a suspension of young tubercle culture in salt solution. The injection was made into the left ventricle. The animals died in 24 to 86 days with tuberculous deposits in kidneys, iris, lungs, and brain. By special methods the author succeeded in staining preparations from kidneys, lungs, and iris which showed appearances very similar to those characteristic of actinomycosis. Paraffin sections were first treated for one minute with Böhmer's hæmatoxylin, washed in water, and then with Victoria blue. They were next heated over a flame until vapour arose, and afterwards decolorised with hydrochloric acid alcohol. After having been washed with water they were treated for one minute with 4 per cent. aqueous solution of eosin, and then washed again. Next they were transferred to alkaline methylen-blue for 30 seconds; then washed with alcohol until no more eosin was given up, and after this immersed for 5 minutes in water slightly acidulated with acetic acid. They were then immersed successively in water, alcohol, xylol, and balsam. The tubercle bacilli are deep blue, the clubs red, and the rest of the tissue blue-violet.

The formula for the Victoria blue solution is,—Alcohol 90 per cent. 30 ccm., anilin 1 ccm., H<sub>2</sub>O 70 ccm., saturated alcoholic solution of Victoria blue 10 ccm.

For the hydrochloric acid alcohol,—Alcohol 70 per cent. 70 ccm., H<sub>2</sub>O 30 ccm., HCl 1 ccm.

For the alkaline methylen-blue solution,—Saturated solution of lithium carbonate 5 ccm., H<sub>2</sub>O 50 ccm., alcohol 90 per cent. 20 ccm., saturated alcoholic solution of methylen-blue 2·5 ccm.

## (2) Preparing Objects.

**Detection of Protoplasmic Threads in Cell-Walls.†**—The following are the principal points in the method of preparing, staining, and mounting sections of vegetable tissue employed by Mr. W. Gardiner to obtain the results described on p. 206.

The method depends upon the use of two principal reagents, viz. the osmic-acid-uranium-nitrate mixture of Kolossow as a fixative, and safranin as a dye. Thymol water is used as a preservative. In material such as that of young endosperms (e.g. *Tamus communis*), no swelling is required, and the tissue, cut into small pieces, may be both killed and fixed at one and the same time by Kolossow's reagent, and then preserved in thymol water. Where only slight swelling is necessary, treatment with water may precede that of Kolossow's reagent. In certain classes of tissue, where the walls are swollen with comparative ease—such as the ordinary vegetative tissue of *Phaseolus vulgaris*, *Tamus communis*, *Nerium Oleander*, *Salisburia adiantifolia*, &c.—small pieces are killed and swollen in an aqueous solution of picric acid, and then fixed in Kolossow's reagent. Finally, where the tissues are more resistant—e.g. in *Robinia Pseudacacia*, *Prunus Laurocerasus*, *Aucuba japonica*—treat-

\* Deutsch. Med. Wochenschr., 1897, p. 653. See Zeitschr. f. wiss. Mikr., xiv. (1897) pp. 413–5.

† Proc. Roy. Soc., lxii. (1897) pp. 102–4.

ment with picric acid may be followed by more severe swelling by means of zinc chloride or sulphuric acid.

With regard to staining, it is possible, in certain special cases, to stain the connecting protoplasmic threads either with safranin alone, or by introducing safranin by means of a somewhat intricate substitution method, the sequence being Hofmann's blue (or soluble water-blue), methylen-blue, safranin. The safranin may then be succeeded by gentian-violet or by eosin; with gentian-violet Gram's method is the best. In all cases the staining is practically limited to the protoplasmic threads.

But with ordinary tissues this method is not applicable; since the whole of the wall becomes deeply stained, and the threads are no longer visible. To avoid this, the method was adopted of staining and washing out, using for the purpose orange G or acid fuchsin. With ordinary tissue the staining appears to be more easily accomplished than with the thick mucilaginous walls of endosperm-cells. Excellent results may be obtained by staining at once with safranin and washing out with orange G. This may be followed by staining with gentian-violet, succeeded by treatment with acid fuchsin, or the sequence of staining may be safranin, gentian-violet, acid fuchsin. Substitutions in which safranin, gentian-violet, and eosin are included give good results.

**Use of Permanganate in Microtechnique.\***—M. M. Tswett finds potassium permanganate a useful reagent for causing swelling of the protoplasmic structures, and thus assisting in the observation of the structure of the chromatophores. The same reagent may also be employed as a macerating substance; beautiful preparations were thus obtained of the sieve-tubes of *Vitis*.

**Preparation of Pigments for Depicting Microscopical Preparations.†**—Herr W. Baklanoff rubs up anilin pigments in a mortar with strong gum arabic solution until the mixture is of the consistency of paste. Glycerin in the proportion of 1 drop to 1 ccm. of the mixture is then added. The paste is then incubated at 37–38° until hard. In this way pigment-masses are made which, when used for depicting microscopical preparations, reproduce the original colours very faithfully. Pigments made in this way form masses which are compact, homogeneous, and do not run or soak through the paper. Hæmatoxylin may be prepared in a similar way.

**Visibility and Appearance of Unstained Centrosomes.‡**—Prof. E. Ballowitz maintains that centrosomes are more easily examined and clearly seen in the unstained condition when merely fixed with Flemming's solution than when treated with sublimate and specific stains.

**Clearing Vegetable Sections.§**—Mr. W. Kirkby recommends the following procedure for treating sections of vegetable tissue, as it leaves the sections in a condition suitable for mounting in liquid, gelatinous, or resinous media:—Place the sections in a fresh clear solution of chlorinated lime until they are quite bleached (2–5 minutes). Warm gently in a test-tube for a few seconds, then quickly replace the solu-

\* Bull. Lab. Bot. Univ. Genève, i. (1897) pp. 13–5.

† Zeitschr. f. wiss. Mikr., xiv. (1897) pp. 366–8.

‡ Tom. cit., pp. 355–9.

§ The Microscope, v. (1897) pp. 151–2.

tion with distilled water, boiling for 2-3 minutes; repeat the last treatment thrice. Wash with 1 per cent. solution of acetic acid, and finally with distilled water. The sections are then quite ready for staining. The author advises alkaline glycerin as a mounting medium, the mixture recommended being composed of glycerin 2 oz., distilled water  $1\frac{1}{2}$  oz., solution of potash (B.P.)  $\frac{1}{2}$  oz.

**Preparing Central Nervous System.\***—Mr. H. J. Berkeley, when studying the lesions produced by the action of certain poisons on the cortical nerve-cell, adopted the following method of preparing and examining the brains. The cerebra are hardened in Müller's fluid until the tissue is of sufficient consistency to admit of fairly thin sections (about two weeks at room temperature). Pieces not more than 3 mm. thick are then immersed in a mixture of 3 per cent. solution bichromate of potash 100 parts, and 1 per cent. osmic acid 30 parts, for 2 or 3 days. On removal, the pieces are mopped up on blotting-paper, washed for a few moments in weak silver nitrate, and then transferred to a solution composed of 2 drops of 10 per cent. phospho-molybdic acid to 60 ccm. of 1 per cent. silver nitrate solution.

This last solution must be prepared the moment before placing the brain tissue in it, and the pieces remain therein for 2 or 3 days; if longer, a few drops of nitrate of silver solution must be added to prevent precipitation. Light does not affect the process unfavourably, though it is better to keep the jars covered up. In winter the solution should be kept at a uniform temperature of about 25° C. By this procedure the individual details of the component parts of the neuron are finer than in Golgi sections; each element stands out clearly and distinctly; the axons and their collaterals are clear, and not too numerously tinged; and the gemmule on the protoplasmic processes are fully and equally impregnated, and appear in their proper relations to the parent dendrite.

**Apparatus for Rapidly Fixing and Hardening Tissues.†**—Prof. R. Thoma describes a simple apparatus made of tin-plate for rapidly hardening pieces of tissue, provided that their structure is not too fragile or delicate. It consists of an overshot wheel, the interior of which has six compartments for preparation glasses. The latter are fixed tightly by means of cotton-wool packing. The wheel is driven by water from a dropping apparatus capable of holding 10 litres of water. The author mentions a dropper of his devising which can be fitted on to the ordinary water-tap.

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

**New Microtome.‡**—The instrument invented by Dr. S. Yankawer consists of two parts, a stand and a movable right-angled piece. The stand is a triangular piece of metal across the base of which an oblong piece of glass is fixed. At the apex of the triangle is *a*, a small elevation with an excavation  $\frac{1}{8}$  in. in diameter and  $\frac{1}{8}$  in. deep. The bottom of the hole is on a level with that of the glass plate. One arm of the sliding piece *e*, *f*, *g*, is  $7\frac{1}{2}$  in. long, the other  $12\frac{1}{2}$  in. long.

\* Johns Hopkins Hospital Rep., vi. (1897) pp. 1-108 (15 pls.).

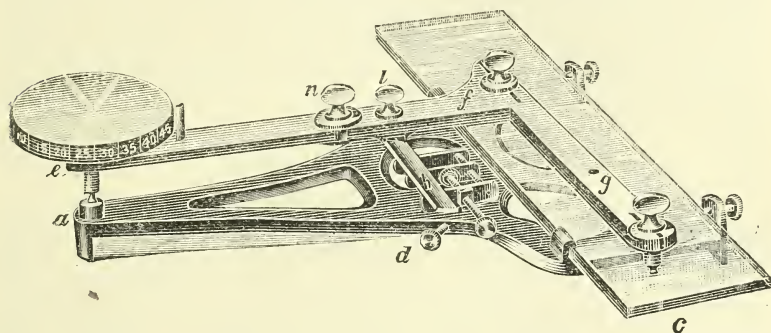
† Zeitschr. f. wiss. Mikr., xiv. (1897) pp. 333-4.

‡ The Microscope, v. (1897) pp. 145-8 (2 figs.).



The angle and both ends are perforated for capped interchangeable screws, each having ten turns to the inch, one of these being the micrometer screw. The blade, fig. 40, *h*, is fastened to the long arm by the screw *l*, so that its edge is parallel to the short arm *fg*. The sliding piece moves along the glass plate, carrying the knife over the specimen to be cut, and the knife is lowered by turning the micrometer screw. The

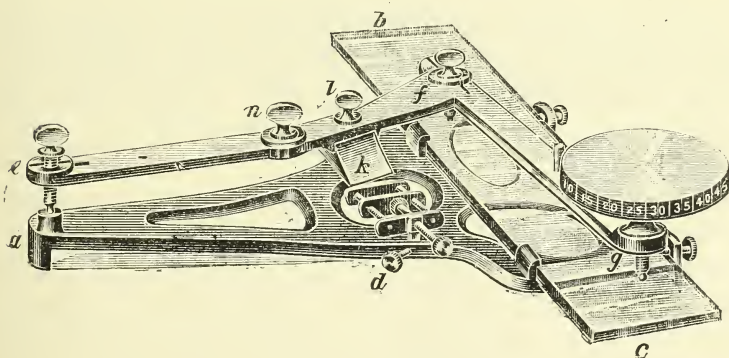
FIG. 40.



thickness of section is obtained as follows: as one turn of the micrometer screw lowers the long arm  $1/10$  in., and as the distance between knife and short arm is one-fifth the length of *ef*, the knife is lowered  $1/50$  in. for a complete revolution. Hence one division of the micrometer screw represents  $1/5000$  in.

By bending the long arm at the joint *n* the knife can be placed in position to cut specimens from  $1/2$  in. upwards. As the knife always

FIG. 41.



moves in a plane parallel to that of the glass plate, the sections are of even thickness and are not wedge-shaped.

For cutting frozen specimens or specimens imbedded in paraffin, the edge of the knife must be placed perpendicular to the direction of the motion. For this purpose a small blade, fig. 41, *k*, is used, and the



position of the screws arranged as in fig. 41. From what has been said, it will be gathered that the microtome is well adapted for celloidin sections, which may be cut as thin as 10 micromillimetres.

**Groot's Improved Lever Microtome.\***—M. J. G. de Groot states that the advantages of his recently improved microtome are that the instrument is very strong; the knife cannot wobble, and may be fixed at any angle. Movement is imparted by the oscillation of a lever. The paraffin block slides easily and smoothly under the knife; it is easy to fix or unfix the object. The thickness of the section is easily regulated. The size of the section may amount to 4–5 cm. square. Celloidin sections are also easily cut by this machine; it is only necessary to immerse the block in paraffin so that it can be fixed to the object-holder in the usual way.

#### (4) Staining and Injecting.

**Chrome-silver Impregnation of Formalin-hardened Brain.†**—Dr. J. S. Bolton has obtained excellent results from brains hardened in formalin for periods of 5 weeks to 12 months according to the size. Pieces of cortex 1/8 in. thick with a base of about 1/4 in. are then immersed in 1 per cent. ammonium bichromate for a few hours to five days. The pieces are then rinsed in distilled water and placed in a bath of 1 per cent. silver nitrate for 16–24 hours or even longer. The pieces are next hardened by immersion in 60 per cent. alcohol for a few hours, and having been mopped up with blotting paper, imbedded without soaking in paraffin. The sections are placed successively in methylated spirit, absolute alcohol, chloroform and xylol, and mounted in xylol balsam without cover-slip.

The author also states that for some time he has passed the Golgi sections into water and developed and fixed them by the method of Kallius, afterwards treating them as above and mounting under a cover-slip.

**Staining Flagella of Bacteria with Orcein.‡**—Prof. Bowhill stains flagella in the following way. Two stock solutions are required, one a saturated alcoholic solution of orcein (the solution improves by keeping for about 10 days); (2) a 20 per cent. solution of tannin. When required for use the foregoing are mixed in the following way:—15 ccm. of orcein solution, 10 ccm. of tannin solution, and 30 ccm. H<sub>2</sub>O, and then filtered.

Young agar cultures are recommended as affording the best results. The film is prepared in the usual way from a suspension in distilled water, and the cover-glass dropped film side downwards on the orcein solution in a watch-glass. The fluid is then gently warmed, and the preparation allowed to float for 10–15 minutes. The cover-glass is then washed and examined in water. If satisfactory, it is dried and mounted in balsam. The bacteria are stained a bluish-purple colour, the flagella being of a somewhat paler hue. A list of nineteen bacteria stained by this method is given, among them being *Sp. cholerae asiaticæ*, *B. typhi abdominalis*, and *B. coli communis*.

\* Ann. Soc. Belge de Microscopie, xxii. (1897) pp. 77–80 (1 fig.).

† Lancet, 1898, i. pp. 218–9.

‡ S.A. Hygienischen Rundschau 1898, No. 3.

**Staining Blood of Oviparous Vertebrata.\***—Dr. E. Giglio-Tos recommends the following method for staining blood-films. The film should be dried quickly in the flame and immediately stained. The stain recommended is a saturated aqueous solution of methylen-blue B.X. One or two drops are placed on the film and left there for one minute. The preparation is then washed with distilled water, afterwards covered with a cover-glass, and luted with olive oil. In this way the preparation will keep for four or five days, and the results for observation are excellent. Attempts to make permanent preparations by means of glycerin and resin were failures.

**Permanent Stain for Starch.†**—Prof. G. Lagerheim describes the following method for imparting a permanent brown stain to starch-grains. The material is first fixed with alcohol, and should it contain chlorophyll, must be left in the spirit until colourless. Eau de Javelle may be advantageously substituted for alcohol, as that fluid rapidly destroys the protoplasm of the cells, leaving the starch-grains intact. The specimen, having been washed, is placed still wet on a slide, and then treated with an iodine solution of the following composition,—water 15 grm., potassium iodide 1·5 grm., iodine 0·5 grm. One drop or so of this solution usually suffices to stain the starch-grains blue. The preparation is next washed with distilled water until the cell-membranes and the plasma have lost their iodine staining, and is thereupon treated with one or more drops of a solution of nitrate of silver (? strength). The silver iodide precipitated in the starch-grains is now reduced by a developer of the following composition,—distilled water 100 grm., sodium sulphite 10 grm., hydrochinon 2 grm. To one cubic centimetre of the developer is added a drop of 10 per cent. solution of potassium carbonate; and the preparation is treated with a few drops of this freshly made solution immediately after it has been washed with distilled water. The preparation now gradually becomes brown, and is mounted in glycerin.

A brown staining of the starch-grains is also obtainable by treating the preparations, after the iodo-potassic iodide solution, with 1 per cent. solution of palladium chloride for a few minutes, and then carefully washing with water.

**Injection Mass.‡**—Herr O. Fränkl uses a mass for injecting the kidney of frogs which was prepared of the following ingredients and in the following manner. Ten to fifteen plates of white gelatin were soaked in water for 24 hours; the superfluous fluid was then poured off, and the mass boiled with an equal bulk of glycerin, and then, after the addition of 4–5 ccm. of sublimate water (concd.), was filtered. The mass, stained with 1–20 Berlin blue or 1–20 carmin, is injected warm. It keeps well if a thymol crystal be added.

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

**Method of Preserving Algæ.§**—Mr. C. Thorn recommends the following method of preserving algæ for demonstration purposes without shrinking. Fix in Flemming's weaker formula (10 ccm. 1 per cent. osmic

\* Zeitschr. f. wiss. Mikr., xiv. (1897) pp. 359–65. † Tom. cit., pp. 350–2.

‡ Zeitschr. f. wiss. Zoologie, 1897, p. 63. See Zeitschr. f. angew. Mikr., iii. (1897) p. 265.

§ Bot. Gazette, xxiv. (1897) p. 373.

acid, 10 ccm. 1 per cent. acetic acid, 25 ccm. 1 per cent. chromic acid, 55 ccm. distilled water). Next drop 10 per cent. glycerin directly into the fixative, each drop being allowed to diffuse before adding more. Continue adding drop by drop until enough glycerin has been put in to cover the specimens when evaporated. The fixative and water should now be allowed to evaporate in a watch-glass where a large surface is exposed. The specimens may now be handled with a needle or knife, and arranged on the slide under a dissecting Microscope. A drop of pure glycerin or of glycerin-jelly makes a very satisfactory mount. Glycerin-jelly has to be used very carefully, but it is the more satisfactory when it can be used with success. The method was found especially useful for demonstrating swarm-spores and the formation of zygospores.

**Media for the Study of Diatoms.\***—Dr. H. van Heurck, in an excellent description of the media used for mounting diatoms, divides these media into resinous and chemical. Of the first, three are discussed, viz. Canada balsam, now but little used, storax, and liquid amber. Storax should be purified by dissolving in chloroform and then filtering it. Liquid amber is prepared by heating the raw material in a water-bath together with equal parts of benzin and absolute alcohol. The solution is filtered and evaporated down until the mass becomes slightly brittle at about 10°. The mass is then redissolved in the same solvents. The chemical media are monobromide of naphthalin, iodide of methyl, and Smith's arsenical fluid. As monobromide of naphthalin dissolves most resinous bodies, the preparation must be closed with strong liquid glue. Its refractive index is 1.658, and hence its visibility is 22. This latter is calculated by taking the difference between the index of the silica of the diatoms, 1.43, and the refractive index of the medium.

Iodide of methyl has a refractive index of 1.743, and therefore a visibility of 31. If saturated with sulphur its index is raised to 1.787.

Prof. H. L. Smith's arsenical medium has an index of 2.4, and consists of realgar dissolved in bromide of arsenic. This medium is prepared by melting together in a retort 1 part of sulphur and 1.7 parts of arsenious acid, and raising the temperature until the product distils. The realgar thus obtained is, together with some tribromide of arsenic, also obtained by distillation, heated in a test-tube, the resulting liquid being of a syrupy consistence and of a greenish-yellow hue. The diatoms, fixed to the cover-glass, are covered with a drop of the medium and then placed on the slide. The slide is then heated over a spirit-lamp until the bromide of arsenic is volatilised. When the gas bubbles cease to be given off, the heating is stopped and the preparation allowed to cool slowly. During the heating the colour of the medium is red, but as the slide cools it becomes yellow.

This method is subject to two grave objections: the medium rapidly deteriorates even in sealed tubes, and two-thirds of the preparations spoil without any obvious reason. The best way to preserve the preparations is to make them when the air is dry and the weather sunny. The slides and cover-glasses should be warm when the medium is applied. The manipulation should be quick and the cover-glass ringed round at once. The slides should be kept in a dry place.

\* Zeitschr. f. angew. Mikr., iii. (1898) pp. 285-97.



**Limpid Colourless Solution of Copal.\***—Dissolve 1 part of gum camphor in 12 parts of sulphuric ether, and to the solution add 4 parts of gum copal. Allow to stand until the copal is thoroughly softened, then add 4 parts of absolute alcohol and  $\frac{1}{4}$  of a part of rectified turpentine oil, and shake well. Set aside for a few days, agitating occasionally the while; in about a week the liquid will have separated into two layers, the upper being a limpid colourless solution of copal. Decant or siphon off the upper layer, and then treat the residue with more alcohol and oil of turpentine to obtain another limpid layer, and so on. If the solution be too thin it can be easily thickened by evaporation. The liquid can be made to dry more slowly by the addition of a small amount of Canada balsam.

#### (6) Miscellaneous.

**Sources of Error in the Plankton Method.†**—Mr. C. A. Kofoid has determined that the ordinary method of collecting plankton by drawing a net made of silk bolting cloth vertically through the water is subject to error, owing to the leakage of organisms through the openings in the silk. The leakage has hitherto been minimised or ignored, and the author has made experiments to ascertain the amount of the escape. The leakage has been tested by means of the Sedgwick-Rafter sand-filter; by hard pressed filter-paper; by the centrifuge; and by the Berkefeld filter.

It was found that in a general way the order given is that of increasing efficiency. The silk retains from 5 per cent. to less than 0.1 per cent. of the total number of the organisms present (excluding bacteria), as contrasted with the catch of the Berkefeld filter. Examination of the sand filtrate showed that 40 per cent. to 65 per cent. of the organisms present were captured, while the filter-paper method yielded 75 per cent. to 85 per cent. of the planktons. By the aid of the centrifuge 98 per cent. were sometimes captured. The Berkefeld filter method was found to be very satisfactory as far as the catch was concerned, but subject to a serious drawback, viz. the presence in considerable amount of infusorial earth, owing to the softness of the filter.

**Method of Demonstrating Assimilation.‡**—Prof. F. Darwin has adopted the following modification of Farmer's method for demonstrating assimilation in green leaves. A leaf of *Elodea canadensis* is mounted in water, and the preparation sealed by carefully ringing it with "wax-mixture" (15 parts resin, 50 parts vaseline, 35 parts bees-wax). The preparation is then placed in the dark, and the observer waits until the available oxygen has been absorbed and the circulation has come to rest, which takes place in from 3 to 6 hours. It is easy to show that the cessation of circulation is due to want of oxygen, by lifting the cover-glass with a needle and adding a drop of fresh water, when the protoplasm will begin to stream in a few minutes.

**Logarithmic Plotting of Biological Data.§**—Mr. D. J. Scourfield suggests that for plotting certain classes of data, such as those connected

\* National Druggist, xxvii. (1897) p. 371.

† Science, vi. (1897) pp. 829-32.

‡ Proc. Cambridge Phil. Soc., ix. (1898) pp. 338-40.

§ Journ. Quekett Micr. Club, vi. (1897) pp. 419-23 (1 pl.).



with the development of the lower animals and plants, the variation in the number of plankton organisms, &c., biologists should use sectional paper ruled logarithmically, or should use the ordinary sectional paper as if the distances at which the lines are drawn represented the logarithms of numbers, and not the numbers themselves. For most purposes it is sufficient if the paper be ruled or taken logarithmically in one direction only, the ruling in the other direction proceeding arithmetically as usual.

Logarithmic plotting has two great advantages over the ordinary method, viz.:—(1) it allows of the graphic representation of an enormous range in the numbers dealt with; and (2) it shows the same proportionate changes by lines having the same angle of slope, which implies that similarly shaped curves denote the same relative course of events. Some data relating to six species of diatoms, a rotifer, and two species of Entomostraca, taken from Apstein's 'Das Süßwasserplankton' are plotted on the plate accompanying the paper in illustration of the method.

**Aitken's Dust-Counter.\***—Dr. A. Macfadyen and Mr. J. Lunt describe the apparatus devised by Dr. Aitken for counting particles of dust in samples of air. The dust particles are rendered visible by supersaturating the air with vapour, whereby each particle becomes a centre of condensation. The essential parts of the apparatus are a metal box, in the top of which is a pair of biconvex lenses, and in the bottom a micrometer-plate etched into squares. The box is lined with bibulous paper moistened with water. The bottom of the box has a couple of perforations, one on each side of the micrometer-scale. Into one is fitted an air-pump, and into the other a tube supplied with three taps, which regulate the quantity and quality of the air allowed to pass into the box. The cooling of the vapour is effected by the air-pump, which rarefies the air. If no dust be present no dew-drops fall, while, when dust-laden air is allowed to enter, a fine rain of dew-drops is deposited on the micrometer. Though extremely ingenious and of great scientific value, the instrument does not appear to be of much practical use, at present at least, for hygienic purposes.

**Enumeration of Blood-Platelets.†**—Drs. T. G. Brodie and A. E. Russell have made an elaborate series of experiments to ascertain the best way to enumerate correctly the number of platelets in a given quantity of blood. The chief difficulties in this examination are due to the fact that the platelets have a great tendency to stick together, and that the red corpuscles become invisible from the action of reagents. The method adopted by the authors was to obtain blood from the finger, either puncturing through a layer of the diluting fluid, or dropping the blood into a glass vessel containing the fluid. The fluids selected after numerous trials were solutions of dahlia in strong glycerin or diluted glycerin; but excellent results were obtained from equal parts of glycerin saturated with dahlia and 2 per cent. NaCl. These solutions have the disadvantage of dissolving out the hæmoglobin from the red-cells, so that the stroma eventually becomes invisible; but as this action takes some time, it is easily possible to complete the enumeration. By adding

\* Trans. Brit. Inst. Preventive Med., 1st series, 1897, pp. 142-51 (1 pl.).

† Journ. Physiology, xxi. (1897) pp. 390-5.

oxalates ( $\text{Na}$ ,  $\text{K}$ ,  $\text{NH}_4$ ) it was also found possible to stain the stroma. A great many other solutions were tried, but for these the original should be consulted. In performing the manipulation it is necessary that the blood should flow freely, and in all cases it must be ascertained that the platelets are distributed uniformly over the field of the Microscope, and that they do not form groups or clumps. The results the authors obtained are that the ratio of the platelets to red corpuscles is as 1 to 85, or 635,300 per cubic mm.

**Cleansing of Slides and Cover-Glasses.\***—Dr. Emma L. Billstein advocates the use of acid alcohol for cleaning slides and cover-glasses. The mixture recommended consists of alcohol 70 per cent. 99 ccm., and hydrochloric acid 1 ccm.; in this the glasses should be immersed for about 5 minutes, transferred to plain alcohol, and then dried.

For removing anilin stains the authoress uses "silver ends," a fossil earth, sold in the form of a greyish-white powder. This mixed with water makes a turbid solution, alkaline on reaction and soapy to the touch, which cleans quickly and imparts a brilliant polish. The glasses should be afterwards washed in water or spirit, to prevent the possibility of any of the microscopic fossils adhering to the surface.

**Cleaning Used Slides.†**—Herr A. Zielina cleans used slides in the following way. After the cover-glasses have been removed, the slides are left until the balsam is dry, and they are then placed in water for a few days. The balsam is then scraped off with a smooth piece of wood, and the slides washed and dried with a cloth.

**Glass-Blowing and Working.‡**—This little volume by Mr. T. Bolas is intended for amateurs, experimentalists, and technicians, and is based on a course of lecture-demonstrations delivered by the author, whose aim has been to make the present work a laboratory and workshop guide to the various phases of glass-working at the blowpipe. Amongst those who will find the instructions useful is the Microscopist. In the little volume will be found descriptions of the necessary tools and utensils and how to work them, the various methods of making glass of different composition so as to be suitable for special purposes, the methods for working and blowing glass, how to colour and modify materials, &c., and remarks on the disintegration and decay of glass.

\* The volume concludes with a chapter on the bibliography of glass; and this contains a short list of works which are of interest either from intrinsic or historical importance or from their ready accessibility.

\* *Mic. Bulletin*, xiv. (1897) p. 45.

† *Zeitschr. f. wiss. Mikr.*, xiv. (1897) pp. 368-9.

‡ Dawbarn & Ward, London, 1898, 212 pp. and 104 figs.

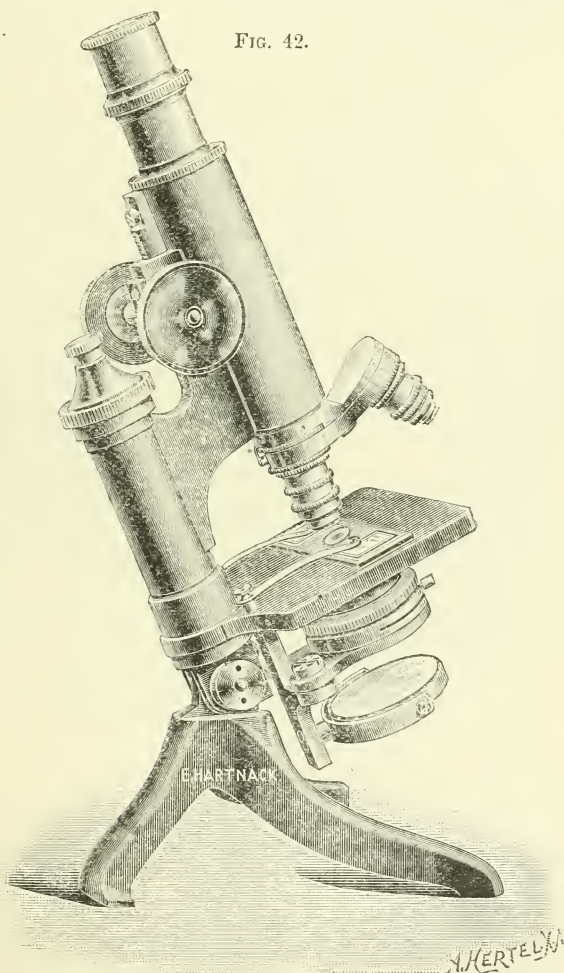
## MICROSCOPY.

## A. Instruments, Accessories, &amp;c.\*

## (1) Stands.

**New Hartnack Microscope.**—The well-known Potsdam firm have brought out a large model Microscope (numbered iv C in their Catalogue) which is represented in the accompanying figure. It has the novelty of

FIG. 42.



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



an iron tripod foot after the English style, and the illuminating apparatus is worked, not by means of a screw, but by freehand motion. The iris diaphragm is applied by a side movement, and consequently affords a better control of its opening. A movable and rotatory stage can be fitted if desired.

**Brugnatelli's Large-size Mineralogical and Petrological Microscope.\***—The stand (fig. 43) is 40 cm. in height, is mounted on an iron horse-shoe foot, and possesses perfect stability at all inclinations. The illuminating arrangement can be raised or depressed at will by means of rackwork. The polariser and condenser are centered by two screws and a spring. A large Nicol's prism is mounted in a rotatory tube, completely independent of all other movements; it can be set in all desired orientations, and can be easily removed for cleaning. Immediately below the Nicol is an iris diaphragm, whose movement, regulated by a button, is completely independent of that of the polarising prism, in such a way that the motion of the diaphragm has no effect on the orientation of the prism. A division into degrees and an index gives this orientation; another division allows the opening of the diaphragm to be read in millimetres. This new arrangement is useful for the determination of the indices of refraction after Viola's method. The condenser is composed of three lenses, of which the largest, with very long focus, is united to the mounting of the Nicol, and serves also for observations with parallel light. The means by which parallel light may be exchanged for very convergent illumination is imitated from the Fuess Microscope, but simplified in the sense that the condenser can be easily unshipped without the aid of a special key. The two superior lenses of the condenser, forming a system with large aperture angle, are mounted on a movable arm fixed under the stage. The arrangement for inserting this and taking it away is the same as in the Fuess Microscopes.

The circular stage is 120 mm. in diameter, and bears on its periphery a scale in degrees on argentan (a metal which keeps its white colour). It moves on a conical bronze guide with great precision. Two verniers read to 10'. On the rotatory stage is fixed a micrometric car of very careful construction whose orthogonal displacements are regulated by two micrometric screws each provided with a drum giving, the one, hundredths of a millimetre, the other, 0.04 mm.; two divisions in millimetres give the entire turns. The maximum movement of the car is 20 mm. in each direction. The object-carrier is applied to the car by means of an easily removable clip.

The upper part carrying the tube has been heightened by 4 cm. by means of a massive cylindrical column; this arrangement, coupled with a more extended draw-out of the tube by means of a rack, allows the placing, between the stage and the objective, of accessories of a certain height, such as the stages of Klein, Fedorow, &c. The tube is composed of two parts jointed one within the other. The lower extremity bears a revolver for three objectives, centerable on the rotation axis of the stage. By means of always screwing the same objective on the same arm of the revolver, a perfect centering is obtained. The centering ring of the revolver is pierced by an opening of rectangular section orientated at 45°.

\* Bull. Soc. Vaud. Sci. Nat., xxxiii. (1897) pp. 228-30 (1 pl.).



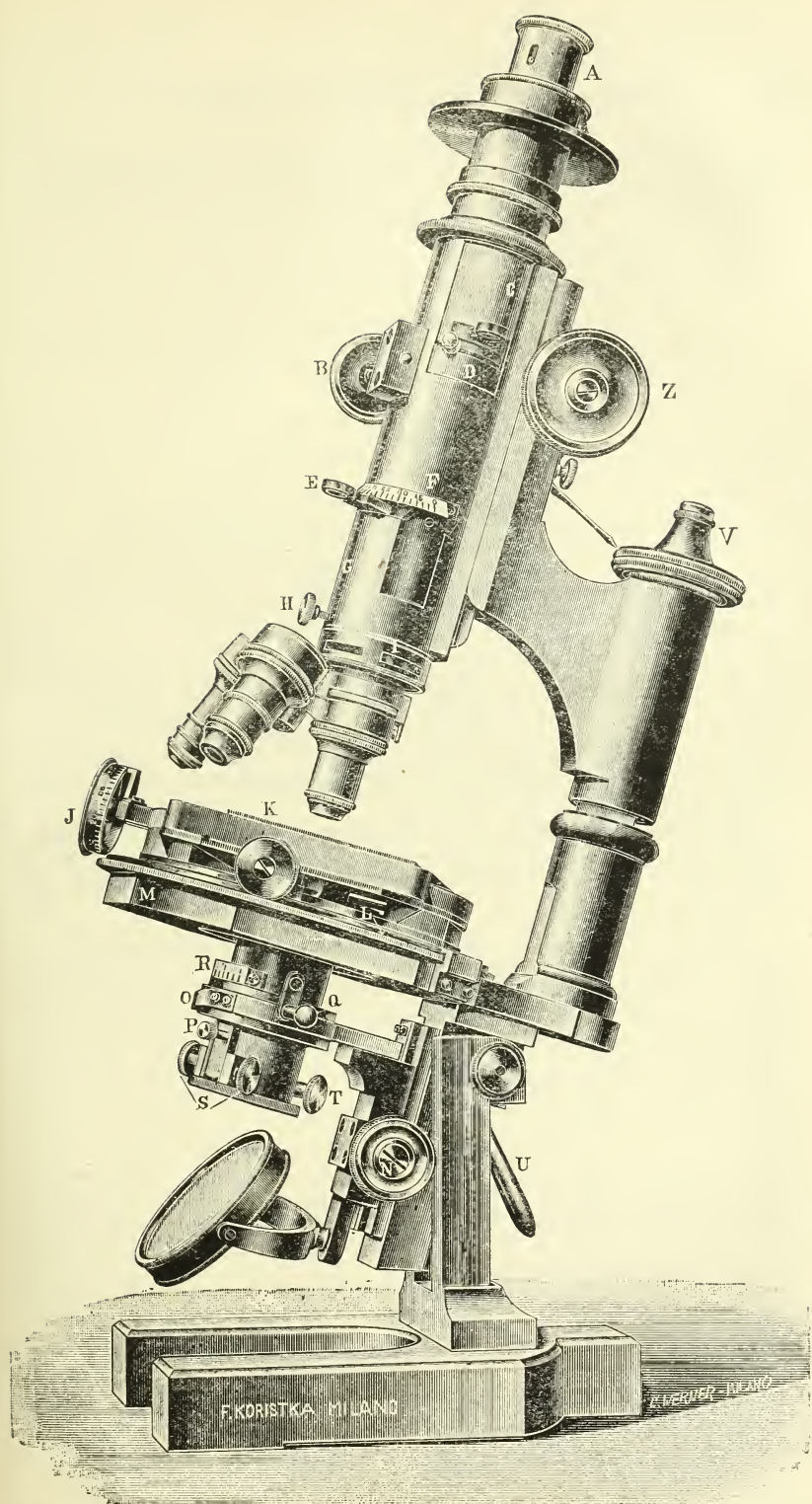


FIG. 43.

and intended to receive the feather edges of gypsum and quartz. This opening can be closed at will by a movable ring. Immediately above this arrangement is a window in the exterior tube, in which slides the analysing Glan-Thompson prism combined with a long focus lens which renders invariable the focal distance of the optical system used with or without the analyser. A new arrangement allows the orientation, by a rotation of  $90^\circ$ , of the analyser; the orientation of the principal section being indicated by an index and a divided dial. All the upper part can be raised or lowered by means of a rack and pinion; the micrometric movement is done by means of a screw whose head is divided into  $\cdot 005$  mm.

In order to use the instrument as a focimeter, a division in millimetres is placed on the lateral part of the rackwork piece, and a vernier below the pinion.

The eye-piece tube is also worked by a rack and pinion, and can be extended about 40 mm. A millimetre scale reads the extension.

In the lower part of the eye-piece tube is an iris-diaphragm opened and shut by means of a button on the left side; directly over this is a window intended for the Bertrand lens to observe the axial figures of interference.

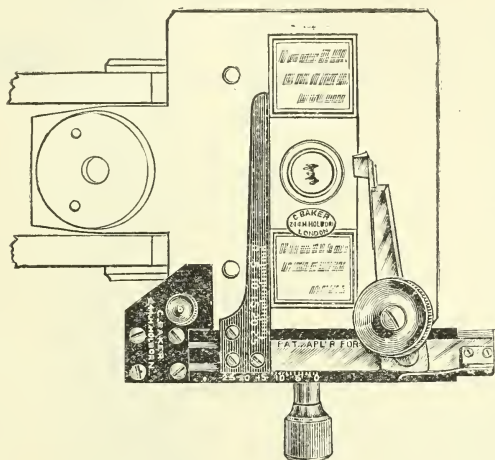
The upper extremity of the tube carries the analyser with divided circle. The mounting of the Glan-Thompson prism is easily removed to introduce the oculars. These are provided with a reticule and a small screw which ensure the invariable position of the crossed threads in relation to the principal sections of the Nicols. The mounting of the upper analyser is also provided with an opening intended to receive the feather edges.

M. Amann, who describes the Microscope, has been able to assure himself, by actual working, that the instrument possesses qualities of high precision, solidity, and simplicity, which render it an eminently practical instrument.

**New Attachable Mechanical Stage.**—Fig. 44 is an illustration of Baker's new mechanical stage, designed by Mr. Allen, and exhibited by Mr. C. L. Curties at the meeting of March 16th last. This apparatus can be easily attached to any Microscope that has a rectangular stage, by means of two thumb-screws; one of these, at the lower end, can be seen in the figure, but the one at the top is not shown, as it is below the stage. This attachment at both extremities gives the apparatus great rigidity. Both rectangular movements are performed by means of rack and pinion. The milled head seen at the right hand causes the transverse arm, upon which the slip rests, to move in a vertical direction over the stage; but the principal novelty in this device is the method by which the transverse movement is controlled. Above the slip a transverse arm, fitted with a rack-and-pinion movement, is pivoted to the vertical dovetailed slide, and to its extremity is fixed a small piece of cork, which is kept in pressure against the top of slip by a spring placed on the right-hand side of the pivot. The friction of this cork pressing on the top of the slip is sufficient to impart transverse movement to the slip, when the milled head is turned. To diminish the friction between the bottom edge of the slip and the lower transverse arm, it is so arranged that the slip bears only on two points. Both

movements are graduated in millimetres for registration as a "finder," and the mechanical movement in each direction is 1 in. Although the transverse movement is 1 in., the examination of a slip is not confined

FIG. 44.



to this amount, as is the case with most mechanical stages, because in this apparatus no limit is imposed as to the lateral position of the slip, neither is the length of the slip limited to the usual 3 in. The device, therefore, becomes eminently useful for the examination of serial sections.

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

**New Hartnack Homogeneous-Immersion Objective.**—The Hartnack firm have brought out a 1/10 in. homogeneous-immersion on the same model as their 1/12 in. (No. 1). Greater experience in the construction of immersion lenses allows the new objective to be issued at a much less price than the 1/12 in. (85 marks as against 125). The numerical aperture is 1.25–1.30; the working distance 0.3–0.35; and magnification with suitable eye-pieces from 280 to 1050 diameters.

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

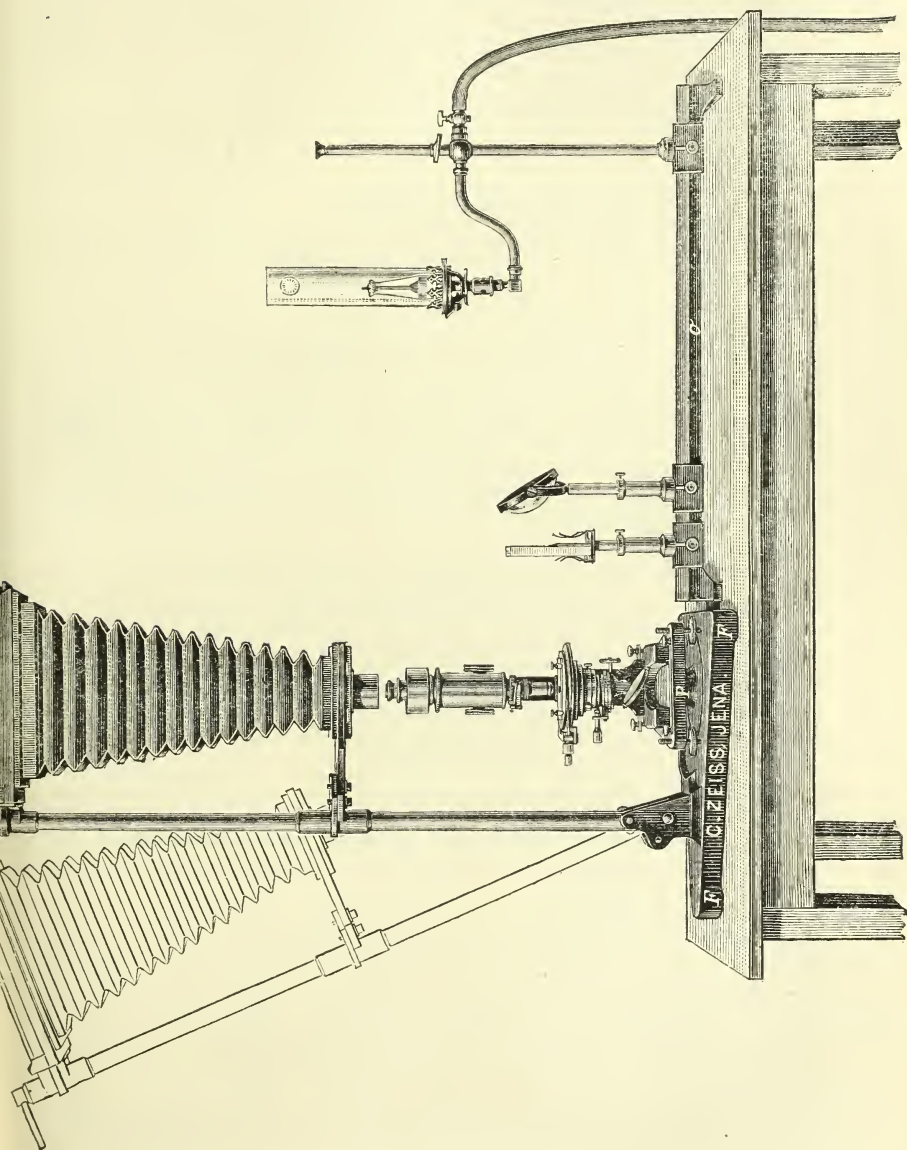
**Zeiss' Combined Horizontal and Vertical Camera.**—This apparatus fully answers to its name, and secures, at the will of the operator, a horizontal, vertical, or 45° inclination of the camera. These three positions will be readily understood from figs. 45 and 46; the outlined portion of fig. 46 showing the 45° position. The massive triangular cast-iron sole plate F has recessed into it a revolving stage P, which is kept in position by a spring, and is adjustable by three setting screws *ii*, so as to give the Microscope its proper elevation and inclination. The Microscope is placed upon the rotating stage, and is adjusted in such a manner that in its vertical position its axis coincides with the axis of rotation of the revolving stage P. This adjustment need only be made once, as a stop







Microscope, together with the revolving stage, may be rotated through  $90^\circ$  in either direction with respect to the central position. The front



set-screws slide both together in a circular groove recessed into the revolving stage; the back set-screws slide in a separate elevated recess. This arrangement, in conjunction with the spring which binds the re-

volving stage and the sole plate together, serves in a very efficient manner to adjust the position of the revolving stage and the Microscope in the two principal directions. A cylindrical guide rod, mounted hinge-fashion in a fork at the rear of the sole plate, supports the camera, either end of which is adjustable by sliding sleeves fitted with thumb-screws. These screws enter with their points into a longitudinal groove running along the rod, and thereby keep the camera from turning round.

A prop at the end of the guide rod maintains the camera in a horizontal position; the vertical position is ensured by a stop at the front of the rod, a bolt at L acting as a clamp. A pin passed through the back of the support obtains the 45° position.

An optical bench adapted to the front of the sole plate provides for the reception of the light-source, condensing lenses, and other accessories.

#### (4) Photomicrography.

**Winkel's New Photomicrographic Apparatus.\***—Dr. H. R. Gaylord, of Dresden, describes this apparatus, which is based on Zeiss' combined horizontal and vertical camera, and differs from it mainly in securing to the operator *any* inclination of the camera he may desire. This is accomplished by attaching the guide rod to a rail which slides in a groove made in the sole plate. The two ends of the camera are connected by stay-rods, which can be clamped at any angle to a sleeve sliding on the guide rod.

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

**Aperture as a Factor in Microscopic Vision.†** (Plates VII.-X.)—Dr. A. Clifford Mercer, in his presidential address before the American Microscopical Society in 1896, describes a long series of experiments undertaken by him with the view of investigating this subject.

Considered theoretically and independently as a factor in microscopic vision, aperture has been almost ignored; although as an associate factor, associated with diffraction by the finer details of microscopic objects, it has received no little attention. In this latter form it is the basis of the Abbe theory of microscopic vision; but the writer wishes to call attention to some unsatisfactory points in the Professor's theory, and to submit a theory of microscopic vision in harmony with an experimental study of aperture.

He believes that the theory of the effect of aperture should be applicable to all projecting lenses (e.g. telescopes as well as Microscopes), explaining resolving power and its limitation; that the diffraction of light by an object should be considered in the same category with other changes in direction in incident light produced by an object, e.g. those resulting from reflection and refraction; that diffracted and others rays leaving an object in changed directions, as well as rays directly transmitted, when travelling the same paths between an object and an objective, are affected alike by aperture; and that the final effects in the image experimentally studied by Abbe are the result of changes

\* Zeitschr. f. wiss. Mikr., xiv. (1898) pp. 313-7 (2 figs.).

† 'An Experimental Study of Aperture as a Factor in Microscopic Vision, Buffalo, 76 pp., 4 pls. and 13 figs.'

ABOVE the objective due to aperture, and not to changes BELOW the objective resulting from diffraction by the finer details of an object.

He first made a preliminary experiment by constructing a gigantic Microscope out of a telescope objective, which had a diameter of  $2\frac{1}{2}$  in. and a focus of 43 in., and an eye-piece which was supported 60 ft. from the objective. No tube was necessary, as the experiment was done in a dark room. The source of light was an electric arc lamp about 27 ft. from the objective. The object was a series of vertical lines scratched with a fine needle-point through the opaque film of an old dry plate negative supported nearly 46 in. in front of the objective. After focusing so as to show the lines through the eye-piece, a plane was found not far from the eye end of the actual telescope-tube, in which was a central image of the electric arc with a series of diffraction images on each side. These images could be dealt with so as to vary the final image seen through the eye-piece, as one deals with the "spectra" at the back of the Microscope objective to produce change in the final image of the ordinary Microscope.

If a telescope objective behaves in the same way as a microscopic objective in experimenting with these diffraction phenomena and associated image changes, does a reason based on such experiments exist for regarding microscopic vision as *sui generis*?

Not only have microscopists noticed in practice the direct relation of aperture to resolution, but also the fact that isolated lines or particles in an object appear broader through an objective of small aperture, and narrower through an objective of large aperture. This narrowing effect of increasing aperture is due to the contraction of the diffraction pattern. *It is easily understood that the projected image discs of a series of close points in an object (or the projected image bands of a series of close lines) might touch or overlap when projected by a lens of small aperture, and, on the other hand, might be separated or resolved when projected by a lens of sufficiently large aperture.*

The separating or resolving power of the telescope is thus explained.

Simple parallel experiments with the telescope and Microscope show that the actual effects of aperture in both instruments are in harmony with the above explanation.

Dr. Mercer now describes 14 experiments, of which the following seem the most important.

*Experiment 1.*—The instrument used was a telescope having an aperture of  $2\frac{1}{2}$  in. and a focus of 43 in., standing 27 ft. from a window in a darkened room. Outside the window was a mirror reflecting light from a bright sky into the room. Of all the light reflected from the mirror that only reached the telescope which passed through two pinholes in a piece of black paper supported in front of the mirror. The diameters of the pinholes were  $1/30$  and  $1/20$  in. respectively, and the distance between them  $1/10$  in. The iris diaphragm of an Abbe substage condenser was supported centrally in a temporary mounting of wood fitting into the hood of the objective. When the diameter of the iris was  $1/16$  in., the two pinholes appeared, when seen through the telescope, as one dim hazy disc. When the diameter was  $1/8$  in., a smaller and more distinct disc was seen. When the diameter was  $3/16$  in., the disc was still



smaller, brighter, and better defined, with a dim, hazy, overlapping disc becoming evident. When the diameter was  $\frac{3}{8}$  in., both discs were brilliant and well separated, their relative sizes and distance apart approaching truth. When the diameter was  $\frac{1}{2}$  in., the picture was more brilliant, the larger disc tending to appear star-like with irradiation. With the full aperture of  $2\frac{1}{2}$  in. irradiation was marked in both. During these observations thin concentric circles of light were glimpsed.

*Experiment 2.*—Instrument and all the conditions same as in experiment 1, except that in the hood was fitted a piece of stiff black paper instead of the iris diaphragm, the circular piece of paper allowing no light to enter the objective except that which passed through a slot corresponding with one of its diameters. Thus the objective was made rectangular in shape, with a narrow aperture in one direction, and a long or wide aperture in the other. The discs seen through the telescope appeared stretched out, as it were, into lines always crossing at an angle of  $90^\circ$ , the diameter of the instrument corresponding with the slot. The width of each line was determined by the long aperture; the length by the narrow aperture. A comparison of the width of each line with its length showed the comparative effect of the two apertures in contracting the diffraction pattern.

*Experiment 3.*—At a distance of 3 ft. in front of a Microscope the same pinholes used in the first two experiments were arranged so as to allow only such light from a lamp flame as passed through them to reach the mirror of the Microscope. The light reaching the mirror was reflected through the substage condenser to an aerial image of the pinhole projected by the condenser in the plane of the Microscope stage. The aerial image of the pinholes was the object observed through the Microscope. Seen with a small diaphragm opening behind the objective, the pinholes appeared as two discs just touching one another. With larger openings the discs became smaller, more brilliant, and separated. The effects of varying aperture (varied by means of diaphragm openings behind the objective) in this experiment with the Microscope were the same as those seen in the first experiment, when aperture was varied by means of diaphragm openings in front of the telescope objective.

*Experiment 4.*—The instrument and all the conditions were the same as in experiment 3, except that a slot corresponding to one diameter was used instead of the central opening in a diaphragm behind the objective. The aperture of the objective thus became rectangular in shape. The image of the pinholes was observed while the slot was turned so as to lie successively in all diameters of the instrument. The effects were the same as those seen when the corresponding experiment 2 was made with the telescope.

*Experiment 5.*—The apparatus and its arrangement same as in experiment 3, with two exceptions. First, for the two pinholes three were substituted, and a group of three parallel slits 2 mm. apart; second, for the diaphragms with a single opening at the back of the objective were substituted diaphragms with two slots. The substage condenser projected these as essentially self-luminous aerial dots and lines in the plane of the Microscope stage. The full aperture image is shown in photo 1, and is most like the original object. Projected with five isolated slots, the image shown in photo 5 differs more; while with



two isolated slots the pictures shown in photos 3 and 4 differ remarkably from the original object.

*Experiment 6.*—The general arrangement of the apparatus and the aerial object remained the same as in the last experiment; but, instead of rectangular slots, annular or zonular slots were used at the back of the objective.

When an annular slot exposed emitting points in a circle 2 mm. in diameter, the image discs were surrounded by rings of light. When the circle was 4 mm. in diameter, the discs were surrounded by closer rings, and the tangential union of diffraction rings between the bands (overlapping discs) resulted in intense lines, in comparison with which the semi-rings at the ends of the bands appeared faint.

Now the diffraction pattern is contracted with every increase in diameter, and this is true in all diameters, and we know that with circular lenses an area of diffracted light varies in extent *inversely* with the square of its diameter or aperture. Therefore the light intensity of such an area would vary directly with the square of the aperture, provided the amount of light transmitted by the objective were to remain the same. But the amount of light transmitted by the objective increases with the square of its aperture. This means that, independently of the contraction of the diffraction pattern, an area of light on the projected image varies in intensity with the square of the aperture of the lens. Increase of aperture, then, adds to the intensity of the diffraction pattern in two ways. The increase of intensity gained in one way must be multiplied by that gained in the other to get the total increase. Thus: *The intensity of the diffraction pattern varies with the square of the square of the aperture.*

*Experiment 7.*—The object of this was to test the conclusion just arrived at. The apparatus and conditions were as in experiment 6, except that for the object was substituted an aerial image of a tiny pin-hole, and for two or more slots behind the objective, single slots were substituted.

Photo 8 is a double photomicrograph of the aerial object. E had the first exposure of 15 seconds, with a slot 3 mm. wide. The aerial object was shifted very slightly to the right. F had an exposure of 1215 ( $= 15 \times 3^4$ ) seconds, with a slot 1 mm. wide. Exposed on the same plate and developed the same, the two images were strictly comparable as to their intensities. The exposures were so timed as to show the first diffraction ring in each case. The triple broadening of the disc by the lesser aperture at once attracts attention, and it is clear that the exposures were as 1 to 81 ( $= 3^4$ ). In the negative it was impossible to say that the two rings differed in intensity, but unfortunately the half tone process has failed to reproduce the rings.

With small apertures the visual pictures of finest particles and lines consist chiefly of diffraction spreading. Such diffraction spreading not only contracts with increasing aperture, but gains in intensity rapidly (with the square of the square of the aperture). Thus, increasing aperture narrows and intensifies most noticeably the picture of finest details. In a similar way, increase of aperture causes diffraction-spreading of boundaries of areas to contract and to approach in intensity that of the area, because that of the former increases with the square of the aperture,

while that of the latter increases with the square of the square of the aperture.

*Experiment 8.*—The apparatus was arranged as in experiment 6, but instead of an aerial image of bright lines and areas, an aerial image of dark lines and areas was photographed. The results in photos 19, 20, 21, and 22 correspond with those in photos 1, 2, 3, and 5 respectively.

*Experiment 10.*—A telescope was arranged as in experiment 2. Instead of the paper diaphragm with a single slot, diaphragms with two or more slots were used in the hood. Emitting points uncovered by pairs of slots (or an isolated zone of aperture) in a telescope behaved as we have seen the corresponding apertures of a Microscope objective behave in the projection of diffracted phenomena.

*Experiment 11.*—Fine and closely ruled lines were observed while diaphragms with minute openings were held between the lines and the eye. The conditions were varied so as to convince one that the dioptric apparatus of the eye projects diffraction phenomena parallel with those previously studied in images projected by the telescope, Microscope, and camera objectives.

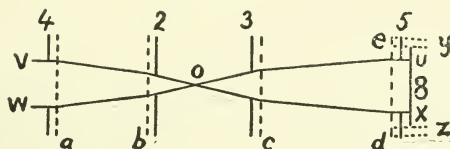
*Experiment 12.*—Photo 32 shows the lines of an Abbe test plate (the same shown inverted in photos 9 and 10), taken when the last emitting surface of the objective used was covered with a diaphragm which had an eccentric opening 1 mm. wide, and transmitted only such rays as had been previously diffracted in the plane of the object by the lines cut through a film of silver. Photo 33 was taken under the same conditions, excepting that the eccentric opening was 2 mm. wide. Photo 34 was taken under the same conditions as photo 32, except that the slot 1 mm. wide transmitted central primary rays. Photo 35 was taken with a slot 2 mm. wide, half central and half excentric, transmitting through its central half primary rays, and through its excentric half diffracted rays. Repetitions of the experiment show that, under parallel conditions, the same aperture gives the same resolution with either diffracted or primary rays. In other words, *Aperture affects diffracted rays from an object as it does primary rays from an object.*

Dr. Mercer now gives at full length his reasons for considering that advantageous reduction in a cone of light between an object and the objective should not exceed, in the case of first-class objectives, one-fourth to one-third (never more than one-half) of the diameter of the cone.

*Experiment 13.*—The general arrangement of apparatus was the same as in taking photo 1 (experiment 5), but instead of the card-holes an opaque card having a cross-shaped hole cut through it was placed against the bull's-eye condenser. An aerial image of the cross was projected in the plane of the Microscope stage by a 1 in. objective arranged as a substage condenser. This aerial image was then observed through a  $1\frac{1}{2}$  in. objective and a 2 in. Huyghenian eye-piece. Let fig. 47 indicate diagrammatically the relative positions of the substage condenser, aerial image, and objective. The dotted line *a* shows the position of the first lens of the substage condenser, and *b* the position of its second lens. Let *c* show the position of the first lens of the objective, and *d* that of the final lens of the objective. Let 4 represent the Powell and Lealand substage diaphragm with circular opening "4" in use. Let the angular

lines V O X and W O U indicate the paths through the lenses which the boundary rays of the light transmitted by the diaphragm opening at 4 travelled. Experimentally it was found that a circular opening 2 mm. in diameter in a diaphragm capping the substage condenser at 2, a circular opening 3 mm. in diameter in a diaphragm capping the objective at 3, and a circular opening 5 mm. in diameter in a diaphragm placed behind the objective at 5, just permitted all the rays transmitted by the diaphragm opening "4" to reach the emitting surface at *d*.

FIG. 47.



Then, without otherwise changing the arrangement, for diaphragm 5 with its opening 5 mm. in diameter was substituted diaphragm 8 (shown just behind 5), which had a zonular opening and an opaque central portion 8 mm. in diameter. Diaphragm 8 obstructed all the primary rays emitted by the lens at *d*. The eye-piece was then removed. On looking at the back of the objective, the zone *cd* uncovered by diaphragm 8 was illuminated, and remained illuminated even when the circular opening at 4 was changed to "1" of the substage diaphragm. The peripheral zone at the back of the objective was illuminated by rays which must have been separated from the direct axial rays at a previously operative lens-surface.

The 2-in. eye-piece was replaced; the eye-lens of the eye-piece was removed, and a photomicrograph taken, as shown in photo 14.

Diaphragm 5 was then exchanged for diaphragm 8, and the photomicrograph taken with the same camera arrangement is shown in photo 17. Immediately afterwards, while observing the image of the annulus on the ground glass as the camera with its 1-in. objective was pushed slowly towards the Microscope, the annulus was seen to shrink gradually and become the small cross shown in photo 18, which is inverted as to the larger cross in photo 14. There can be no doubt then that the annulus was illuminated by light which in some way was derived from the aerial image of the cross projected in the plane of the stage, and the inversion of the smaller image shows that the excentric rays were conveyed to at least one more focus than the direct axial rays. It is probable that the excentric rays were separated from the direct axial pencil by internal and converging reflection at the emitting surface of the front lens of the objective, and returned to the front surface, thence to be reflected back in an onward direction to and through the emitting surface along excentric paths towards the annulus.

The Abbe "spectra" now claim our attention. We have found that they are not indispensable in some of the images of microscopic vision. "Spectra" are images of an opening in the diaphragm of a substage condenser, or of a source of light, formed above the objective in micro-



scopic vision by diffracted rays originating in the object. Can an image of an opening, or of a source of light formed in the Microscope-tube between the objective and the field lens of the eye-piece by diffracted rays, have any influence on the primary ray-image of microscopic vision formed between the two lenses of the eye-piece? Two widely separated images cannot be seen through the Microscope at the same time. In other words, such images cannot be united to form a joint visual picture.

*Dr. Mercer thinks that the Abbe "spectra" are sometimes present as an accident of Microscope projection, and sometimes they are not.*

Why do "spectra" when present appear to be of such importance? The "spectra" are so placed in a plane at the back of the objective that when a slotted diaphragm in their plane uncovers the "spectra," the same diaphragm uncovers simultaneously certain emitting points of the projecting lens. If now one of the slots of such a diaphragm be covered, one of the "spectra" disappears, and a corresponding change occurs in the projected image. But this change is due to the loss of the slot and corresponding emitting point of the projecting lens, and not to the loss of one of the "spectra"; because *if the "spectra" were absent in full cone illumination, the covering the slot in the diaphragm would produce the same change in the projected image.*

When the axial illuminating pencil is narrow and the Abbe "spectra" are separated by well-marked intervals of darkness, the Abbe theory ignores the emitting surface of the objective corresponding with the intervals of darkness. In harmony with this partial neglect of aperture, resolution in the Abbe theory may be said to increase by jumps. So long as a central image of the source of light is to be seen at the back of the objective, resolution is not present. The aperture may be increased without change in the contraction of the diffraction pattern and in accompanying resolution, so long as the central image alone is to be seen at the back of the objective. But the moment the increase in aperture is sufficient to uncover or admit one flanking "spectrum" image, resolution is present. With greater aperture no improvement is to be seen until another "spectrum" image is uncovered or admitted.

On the other hand, with full cone illumination, resolution increases continuously, and not by jumps or by periodic accessions. The portions of aperture neglected in the Abbe theory are effective in full cone illumination. They contribute in proportion to their breadth, radially from the principal axis, to the contraction of diffraction patterns. And thus they may resolve additional finer details (experiment 14) in an object, or increase the distinctness of the resolution of details already resolved.

*Experiment 14.*—A Microscope was arranged to exhibit the lines shown in photos 10 and 35. For the optical part of a Powell and Lealand substage condenser was substituted a Powell and Lealand 1-in. objective. A Powell and Lealand 3-in. objective and a "10 compensating" eye-piece were used. A diaphragm with an opening 10 mm. in diam. was placed at the back of the objective. The revolving diaphragm of the substage condenser was turned so as to bring opening "1" into use. The closer lines of the test plate were resolved. On removing the eye-piece and looking at the back of the objective, a central image of the opening in the diaphragm of the substage condenser was seen,



flanked on each side at the limit of the aperture by about half of an Abbe "spectrum" image. The more distant halves of the "spectra" were just outside the limit of the aperture, and could not be seen.

Then, for the diaphragm with an opening 10 mm. in diam. at the back of the objective was substituted a diaphragm having an opening 6 mm. in diam. The latter just covered both halves of the two flanking "spectra," and left on each side of the central image a breadth of darkness corresponding with one portion of the aperture neglected in the Abbe theory. On replacing the eye-piece and again looking at the test plate, the closer lines could not be seen. Resolution failed, because under the conditions present the Abbe theory requires for resolution the admission, by the diaphragm at the back of the objective, of at least a part of one "spectrum" image in addition to the central image of the opening in the diaphragm of the substage condenser. Again the eye-piece was removed. The diaphragm of the substage condenser was turned so as to bring opening "3" into use. This change caused the central image seen at the back of the objective to increase in size until it filled the opening 6 mm. in diam. in the diaphragm at the back of the objective. On replacing the eye-piece and looking at the test plate once more, the closer lines were seen. Resolution returned as a result of the additional light from the larger opening on the diaphragm of the substage condenser reaching and utilising the portions of aperture which were previously dark under the conditions necessary to the Abbe theory.

Dr. Mercer summarises the results of his experiments thus:—

(1) Diffracted rays leaving an object may be considered in the same category with other rays changed in direction by an object.

(2) The diffraction phenomena seen in a projected image are essentially the effect of changes in light *above* the objective due to a function of aperture, and not to changes *below* the objective due to diffraction of light in the plane of the object.

(3) Diffraction in the plane of the object does, under some circumstances, furnish light to certain parts of an aperture from which primary rays are absent, and thus enables aperture to more fully determine the character of the projected image, resulting in a more nearly truthful image, or, on the other hand, in false appearances. This is the gist of the Abbe phenomena of microscopic vision.

(4) But such phenomena are not peculiar to microscopic vision, notwithstanding Prof. Abbe's claim to the contrary.

(5) With any positive lens similar and more brilliant results may be got by utilising corresponding pencils of primary rays, instead of isolated pencils of diffracted rays.

(6) Still more trustworthy results may be got by using continuous apertures three-fourths (in diameter) full of primary rays instead of the isolated pencils of primary rays.

(7) An advantage peculiar to using narrow cone illumination with an objective of wide aperture (the only illumination admissible in the Abbe theory) consists in giving, under suitable conditions, approximately the acme of resolving power simultaneously in each of several diameters. Thus a circular aperture is approximately squared or made rectangular as to resolving power in several of its diameters simultaneously.

(8) Special attention is called to the fact that the Abbe theory deals with complex objects; for only such objects are subject to resolution. Single particles and uniform areas are outside its domain. These latter, however, are microscopic objects, and all objects are essentially different shaped aggregations of points. An isolated point-like particle, no matter what its minuteness, may be seen if it present sufficient contrast with the surrounding microscopic field. The size of the disc image is no less than a limit determined finally by aperture. That limit in size, varying inversely with aperture, determines the limit of resolving power. This is the gist of the theory of microscopic vision which harmonises with our experimental study of aperture.

**Microscopic Vision.\***—A paper read by Mr. E. M. Nelson under the above title gives an interesting historical sketch of the theory of microscopic vision and its present position.

After glancing at Dr. Goring's experiments on angular aperture in 1837, Mr. Nelson goes rather fully into the history of the controversy initiated by Dr. Pigott in the *M. M. J.* (July 1870). Amid much error and much high-sounding Greek verbiage, Dr. Pigott had the merit of stating several important truths, viz.:—

(1) That a water-immersion lens can have a greater aperture than any dry lens, and similarly a homogeneous than a water-immersion.

(2) That illuminating power is increased by the use of higher refractive media.

(3) The suggestion of homogeneous immersion.

Possibly Dr. Pigott's lucubrations suggested to Mr. R. B. Tolles the improvements connected with his name; for in 1874 he actually constructed a balsam immersion objective, and in 1873 an apertometer on much the same principle as that now known as Abbe's; moreover, the word "homogeneous" as applied to immersion objectives is probably due to Mr. Tolles.

Mr. Nelson now traces the history of diffraction from Fraunhofer, through Herschel and Nobert, to Dr. Barnard in 1869. Fraunhofer, in studying the well-known equation  $\sin \theta = \frac{\lambda}{\delta}$ , had thought that the limit

of microscopic vision was reached when  $\delta = \lambda$  and  $\theta$  consequently equalled  $90^\circ$ . If Fraunhofer's theory were correct, not even the 9th band of Nobert's 19th band test-plate (56,300 lines per inch) could be resolved in monochromatic yellow light; but Dr. Barnard and Colonel Woodward had resolved up to the 19th (112,600 lines per inch) with a P. and L. water immersion 1/16.

We are now brought to the era when the opinions of Abbe and Helmholtz were made known to this country by Dr. Fripp. Abbe's great idea of using Snell's equation (law of sines) as a standard to which all kinds of aperture might be referred, simplified matters, and put fresh meanings into and enlarged the ideas connected with aperture. Both Pigott and Tolles used Snell's law,  $\mu \sin \phi = \mu' \sin \phi'$ ; but Abbe went further, and said,  $\mu \sin \phi = \mu' \sin \phi' = \text{numerical aperture}$ .

Among the enlarged ideas put into the word "aperture" by Prof. Abbe, its photometric value stands first. The radiation of light from

\* Proc. Bristol Naturalists' Soc., viii. pt. ii. (1896-7) pp. 141-66 (6 figs.)

any surface diminishes in proportion to the cosine of the inclination of the rays to the normal. This means that the amount of light radiating from a point in a homogeneous medium varies as  $(\sin u)^2$ , where  $u$  is the semi-angle of the solid cone. The next point is that the radiation of energy, such as light and heat, in different media, varies as  $(n^2)$ , where  $n$  is the refractive index of the medium. Therefore the total effect of radiation in any medium is proportional to  $(n \sin u)^2$ , i.e. the square of the numerical aperture.

The Lagrange-Helmholtz-Abbe theorem may be called the Magna Charta of Microscopy. If  $u$  and  $u'$  be the angles of convergence of any ray on either side of a given system,  $n$  and  $n'$  the refractive indices of the media on either side, and  $M$  the magnifying power, then

Lagrange (1803) showed that  $\frac{u}{u'} = M$ , the media on both sides of the system being the same, and the aperture small.

Helmholtz (1866) that  $\frac{n}{n'} \frac{u}{u'} = M$ , the media different, but the aperture small.

Abbe (1873) that  $\frac{n \sin u}{n' \sin u'} = M$  for any aperture or media.

Mr. Nelson outlines the proof of the last result, and shows that the following useful formulæ may be obtained:

$$(i.) \frac{\frac{1}{2} \text{ back lens}}{N.A.} = f;$$

$$(ii.) \frac{M \times \frac{1}{2} \text{ back lens}}{\text{distance}} = N.A.$$

This gives a simple way of measuring the equivalent focus of any objective without an apertometer. The image of a stage micrometer is projected without an eye-piece on a screen, say 2 or 3 ft. from the back lens, and  $M$  the magnifying power is measured; this, when multiplied by half the diameter of the back lens, and the product divided by the projection distance, gives the N.A.

In discussing the Abbe theory that coarse structures are imaged according to ordinary dioptric laws, and fine ones according to diffraction phenomena, Mr. Nelson points out that the line of demarcation between coarse and fine was placed at  $1/2500$  of an in., but that this position is untenable, because the diffraction pencils from gratings such as wire sieves, linen threads, or ruled scales, where the intervals are at least  $1/50$  in., can be easily seen without any special apparatus. With suitable apparatus the spectra arising from much larger gratings have been made visible.

The following simple but important experiments prove that the Fraunhofer diffraction law applies even to large objects which can be seen without instrumental aid.

(1) When a scale on a carpenter's rule is examined through a diaphragm held close to the eye, the hole being  $0.011$  in. in diameter, some divisions on the rule can just be perceived at  $7\frac{1}{2}$  in. What is the fineness of the divisions?

Let  $a$  be the diameter of the hole, and  $\lambda$  the wave-length, say

1/45,000 in.,  $\delta$  being the value of one line and interspace. Then  $\frac{a}{7.5}$  will be the angle the hole subtends at the distance of the scale. By the Fraunhofer law  $u$ , the divergence of the diffraction beams is such that  $\sin u = \frac{\lambda}{\delta}$ ; but, in order that two diffracted beams may just pass through the hole, which is the condition of the limit of visibility of the grating,  $\sin u$  must also  $= \frac{a}{7.5}$ ; therefore  $\frac{\lambda}{\delta} = \frac{a}{7.5}$ , and  $\delta = \frac{7.5 \lambda}{.011} = 1/66$  in. The scale actually had 64 lines per inch.

(2) The converse problem may be treated in a similar way. Looking through a hole in a diaphragm placed close to the eye, a scale of 50 lines to the inch can be just perceived at a distance of 9 in. What is the diameter of the hole?

The hole here subtends an angle of  $\frac{a}{9}$  at the scale; this angle must be equal to  $\sin u$  if the grating is just resolved. Therefore  $\sin u = \frac{\lambda}{\delta} = \frac{a}{9}$ ;  $a = \frac{9 \lambda}{.02} = .01$  in. The actual size of the hole by micro-metric measurement was .011 in.

These experiments show that the diffraction limit is at least fifty times greater than that assumed, and the line of demarcation between fine and coarse must be altered to 1/50 in., from which it follows that the only Microscope images to be accepted as truthful are those of objects larger than the 1/50 in., a conclusion which is known to be absurd.

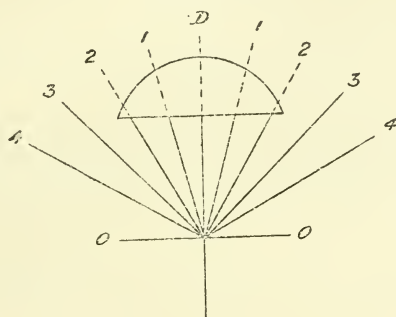
Another dangerous conclusion from the diffraction theory was that, because the image of fine structure depends upon spectra, therefore make spectra. This meant that the spectra should be made as bright as possible by reducing the aperture of the illuminating cone. Reducing the aperture of the illuminating cone is analogous in its effect to reducing the slit of a spectroscop. Narrow the illuminating beam to a mere point, and the spectra from any diatomic structure will become brightly coloured. (So much is this the case that if a spectroscop is not at hand, a coarse diatom, such as a *Pinnularia*, illuminated by a narrow cone when a suitable objective is employed, makes a very good substitute; and a light-filter of any kind can be tested by examining the spectra at the back of the objective.) Prof. Abbe, unfortunately, says that there is not the least ground for supposing that a broad illuminating beam can be expected to give a truer image than a narrow axial illuminating pencil. Apart from the question of the manufacture of false images, this statement renders unnecessary any improvement in the objective—a statement most damaging to the interests of microscopy.

In discussing these questions Mr. Nelson carefully defines his nomenclature. The central white beam, sometimes called the "Central Maximum," will be called the dioptric beam (D); the first coloured spectrum next the dioptric beam the spectrum of the first order (1); the one next to that a spectrum of the second order (2); and so on; see fig. 48.



The law, then, for the manufacture of the simplest form of false image, is the union of a spectrum of the second order with D, when that of the first order is suppressed. In this case the false image will consist of a doubling of line structures, and the insertion of an intercostal in hexagonal and similar structures. To repeat, the cause of the false image is the suppression of (1) and not merely the admission, *per se*, of

FIG. 48.



(2); for if (2) is admitted and combined with the dioptric beam, (1) being also combined with them, there will be no false image. This effect can be obtained by a suitable stop, or by using a narrow cone. When a narrow axial cone is used, spectra of the first order pass through an intermediate zone of the objective aperture, whilst those of the second order pass through an outer zone (fig. 48); then spherical aberration

FIG. 49.

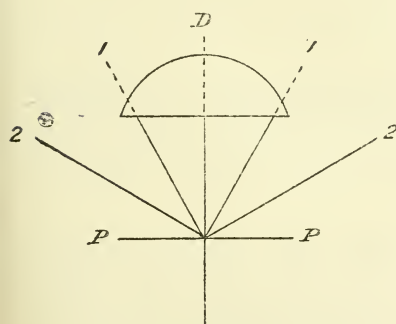
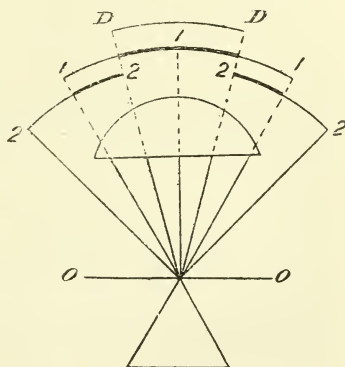


FIG. 50.



(which is always present, even in the best objectives, to a far greater extent than is generally supposed) will cause spectra of the second order to be combined with D, to the exclusion of (1), thereby forming a false image. Putting it in a popular form, it may be said that the eye is quite unable to distinguish whether any given spectra are spectra of

the first order arising from a fine structure (P, P, fig. 49), or spectra of the second order from a coarse structure (O, O, fig. 48); so that, if (2) and D are brought into focus together while spherical aberration is causing (1) to be out of focus, the result is that the eye interprets (2) of the coarse structure (O, O, fig. 48) as if they were (1) of fine structure (P, P, fig. 49), consequently a ghost image of fine structure is seen. If therefore the coarse structure (O, O, fig. 48) which in this instance would be the true image, had a certain number of lines or marks to the inch, say 12,000, then the ghost image would have precisely double that quantity, or 24,000 to the inch.

False images of greater complexity may be made by combining (3) with D, when (1) and (2) are excluded, &c.

All these false images are dispelled by means of the wide angled axial cone of illumination (i.e.  $3/4$  cone), because it causes groups of (1) to pass through the same zone of the objective as those of (2), and unites as well portions of (1) with D (fig. 50), thus rendering their separation impossible.

FIG. 51.

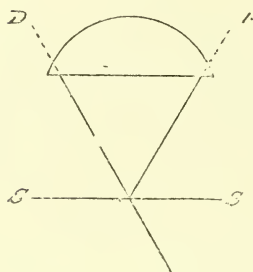
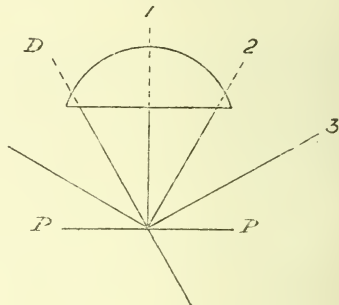


FIG. 52.



In fig. 50, D, (1), and (2) are distinguished by being drawn in steps, the overlapping portions being indicated by thick lines. The structure O, O, is supposed to have 12,000 lines per inch, and to be similar to that in fig. 48. P, P, in figs. 49 and 52, are supposed to have 24,000 lines per inch, while S, S, in fig. 51, have 48,000. In these figures the refraction of the lens has been omitted.

"Oblique illumination" is another form of the small cone, and was probably invented by Dr. Goring in 1826. The dioptric beam passes through a marginal zone on one side; and when S, S, is barely resolved (1) passes through the same zone on the opposite side (fig. 51); duplication is then impossible, and the image will be correct with regard to the fineness (48,000 per inch). If the structure is sufficiently coarse (24,000 per inch) to permit a spectrum of (2) and D to pass through the marginal zone, then a spectrum of (1) will pass through the centre of the objective. Spherical aberration will prevent combination, and therefore an image of double the fineness (48,000 per inch) will be seen.

Tests to determine the reality of the ghosts:—

(1) They usually occur when a small cone of illumination is employed.

(2) They must be an integral multiple of some real structure: thus, if the real structure is 12,000, the ghost may be 24,000, 36,000, 48,000, &c., but it can never be 18,000 or 30,000.

(3) The ghosts invariably have a focus differing from that of the true structure.

The "black and white dot" is a term used to express the fact that when an object—e.g. a siliceous plate—is viewed under the Microscope, its edge assumes either a black or white appearance, according to changes in focus; but when the edge is an inner edge of a hole, and the hole is very minute, the black edge on one side of the hole will meet the black edge on the opposite side, and the hole will appear as a "black dot"; but when the focus is arranged so as to give a white edge, then the hole becomes a white dot. Although primarily applied to diatoms, the term is applicable to all minute microscopical objects, such as bacteria, hairs, flagella, and the edges of objects generally. This phenomenon is found to depend upon the aperture of the objective; for the greater the aperture the easier it is to obtain a black dot. When, however, the hole becomes excessively minute, a black dot is no longer attainable, and we have to content ourselves with the white dot appearance. There is nothing in the theory of microscopic vision, as at present enunciated, to explain why a larger aperture is required to resolve the black than the white dot. In dealing with the limit of microscopic vision, the question arises whether we mean the black or white dot limit, for there must be two limits. Again, which is the more correct picture? Moreover, as these images occur at different foci, which is the correct focus? and as it depends on the adjustment of the objective, which is the correct adjustment?

Another problem awaiting solution relates to dark-ground illumination. This illumination is best obtained by placing an opaque stop at the back of the condenser to stop out an axial cone of greater aperture than that of the objective. It is found in practice that, when the ground is strictly dark, the resolving limit of all objectives is lowered. When the stop at the back of the objective is hardly large enough, the ground assumes a pearly appearance; in this case the resolving limit is at its maximum.

#### (6) Miscellaneous. ;

**Vessel for Treatment of Paraffin Sections with Staining and other Solutions.\***—Dr. L. Buscalioni describes a vessel (figs. 53–57) which is a modification of that devised by D. Caro for the treatment of paraffin sections. The receiver is a rectangular glass jar measuring 8 cm. high, 6.5 cm. broad, and 8.3 cm. long. The lid is made of ebonite and is 8.8 cm. long by 7 cm. broad. In it are twelve openings, each about 4 cm. long, and sufficiently wide to take two slides placed back to back. On the under surface of the lid is a groove for the purpose of fitting the lid on to the receiver. The lid is covered with a cap made of

\* Malpighia, xi. (1897) pp. 458–60 (5 figs.). Zeitsch. f. wiss. Mikr., xiv. (1898) pp. 442–4.



tin or iron plate, and is let into a groove on the upper surface of the lid. This cap or cover is about 1 cm. high, 7.7 cm. long, and 6 cm. broad, and its object is to prevent evaporation of the fluid.

FIG. 53.

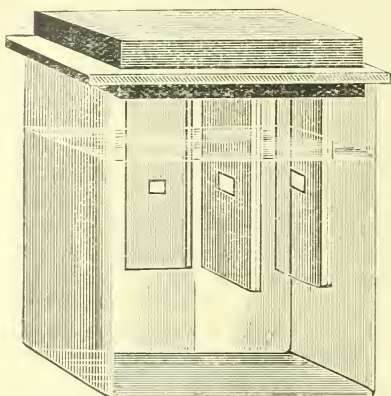


FIG. 54.

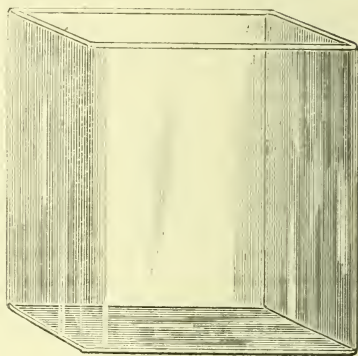


FIG. 55.



FIG. 56.

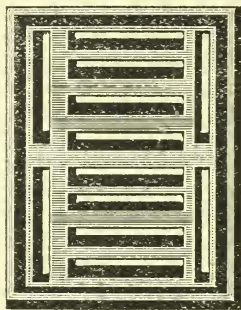


FIG. 57.



The slides are let into the fissures in the lid by placing a couple back to back, and fixing them together by means of a rubber ring. The ring not only holds them together but supports them on the lid.

**New Rapid Filter.\***—Herr E. Funck has devised an apparatus for rapidly filtering nutrient media such as agar, gelatin, &c. The apparatus is made of copper, and consists of the double filter A (fig. 58), having a side tube E for filling the interspace A with glycerin or paraffin.

The outflow tube is provided with a metal disk F, which serves to prevent dust and impurities from contaminating the filtered fluid, by closing G. The cover B of the hopper is fastened by means of screws C, and rendered air-tight through the intermediation of a rubber or asbestos band. The tube D serves to regulate the pressure of the steam

\* Centralbl. Bakt. u. Par., 2<sup>te</sup> Abt., iv. (1898) pp. 200-1 (1 fig.).

and the filtration. Inside the large funnel is a small perforated funnel H which serves to protect the paper filter. The filter may be made of

FIG. 58.

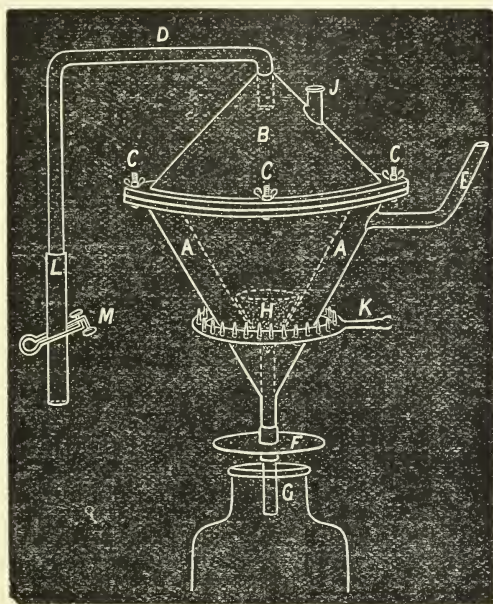


FIG. 59.



thick filter paper or of flannel. The tube J is for the introduction of the fluid to be filtered. The apparatus is supported by the ring K. In order to heat the funnel only a small flame is necessary.

**Injection Syringe for Bacteriological Work.\***—Dr. A. Cantani describes a syringe he has been using for some time past, with excellent effects. The syringe, which is easily constructed, consists of a nozzle, a glass tube, and a rubber ball, similar to that in Koch's syringe.

As may be seen from fig. 59, the front end of the syringe is narrowed a little, and to this is fastened the nozzle. About two centimetres from the other end is a capillary constriction, beyond which the tube is packed with cotton-wool. The ball is joined to the syringe by a piece of rubber tubing *b*. The instrument is very easily made, easily manipulated, and easily sterilised.

**New Autoclave.†**—Dr. F. Abba describes an autoclave which he has used for some time with very satisfactory results. It works up to a pressure of  $1\frac{1}{2}$  atmosphere, giving a temperature of  $112^{\circ}$ . Though

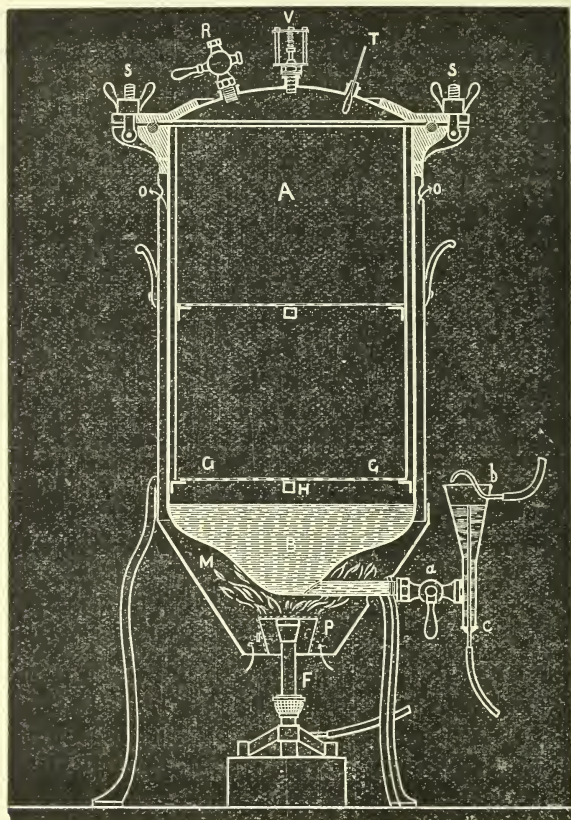
\* Centralbl. Bakt. u. Par., 1<sup>re</sup> Abt., xxiii. (1898) pp. 217-8 (1 fig.).

† Tom. cit., pp. 462-5 (2 figs.).

very effective, the apparatus is much less costly than most sterilisers, as these are made, most unnecessarily, to withstand a much higher pressure.

The apparatus consists of a copper cylinder or jacket A B (fig. 60) tinned on the inside. The lid is fastened down with the screws S, and the interior rendered air-tight by means of an intervening rubber band. The water-holder B is shaped so as to offer as large a surface as possible to the flame. Inside the jacket is the sterilising chamber, divided into

FIG. 60.



compartments by perforated partitions, and supported on fillets. Its diameter is 27 cm., and its height 46 cm. In the lid are three holes, one for a tap R, another for a safety valve V, and the third for the thermometer T. Connected by a pipe *d*, fitted with a stop-cock *a*, is a funnel *b c*. The jacket is enclosed in a metal case with several openings *o*, for the escape of hot air.

The apparatus can be used for sterilising at 100° or at 112°. If the former temperature be desired, the tap R is kept open. If the autoclave



is to be worked at  $112^{\circ}$ , the water-holder is filled up to the level indicated, the tap *a* is closed, and R left open until the thermometer reaches  $98^{\circ}$ – $99^{\circ}$ , a point which indicates that all the air has been driven out of the apparatus. The tap R is then turned, and the temperature soon rises to  $112^{\circ}$ . Should the temperature and pressure rise above this, the steam escapes through the safety valve.

By a preconcerted adjustment of the tap R, the valve V, and the flame F, a temperature constant between  $100^{\circ}$  and  $112^{\circ}$  can be maintained.

### B. Technique.\*

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

**Aseptic Cultivation of Mycetozoa.**†—Dr. C. O. Miller, who described a method for the cultivation of Protozoa,‡ finds that some modifications are necessary for Mycetozoa. They will grow in sterilised dilute hay infusion, or 2 per cent. of milk in water; but for the formation of sporanges it is usually advantageous, and sometimes essential, to furnish the organisms with a mechanical support as a means of getting out of the water. The medium is prepared by putting a handful of hay in a jar and washing it until the water remains colourless. It is then covered with fresh water, and allowed to soak overnight. The following day the water is poured off, filtered, diluted with fresh water until it is of a white wine colour, and 2 per cent. of milk added. It is then filtered, put into a flask and sterilised for future use. The macerated hay is cut, and placed in Erlenmeyer's flasks; the first portion is cut short enough to form a tolerably compact layer at the bottom of the flask to the depth of 1 cm.; the rest is cut sufficiently long to form a very loose layer, reaching about two-thirds the way up the sides of the flask, care being taken not to allow any of the stems to reach the cotton. Sufficient water is placed in the flasks to cover the hay, and they are sterilised for 15 minutes. On the following day fresh water is substituted, and they are again sterilised. The water is once more poured off, and enough of the hay infusion and milk previously prepared is added until it is about 1 cm. deep. The flasks are then sterilised for 10 minutes on three successive days. They are then ready for use.

The cultures are usually transplanted by means of a sterilised pipette.

**Peptonised Milk for the Cultivation of Lactic Acid Ferments.**§—Herr O. Jensen, who confirms the opinion of Kayser as to the excellence of peptonised milk as a medium for cultivating lactic acid bacteria, gives the following method for its preparation.

After the milk is sterilised, 10 ccm. per litre of pure hydrochloric acid and 2 grm. of pepsin are added, and the mixture incubated at  $35^{\circ}$ – $37^{\circ}$ . At first, until the casein is separated and the pepsin dissolved, the flask is to be frequently shaken up, but afterwards this need only be done occasionally.

\* 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. † Quart. Journ. Micr. Sci., xli. (1898) pp. 46–9 (2 pls.).

‡ Cf. this Journal, 1894, p. 744.

Centralbl. Bakt. u. Par., 2<sup>te</sup> Abt., iv. (1898) pp. 196–9.



In 36-48 hours it is neutralised, sterilised, and filtered through paper. The reaction should be about neutral to litmus paper, and 5 ccm. of the peptonised milk should, with phenolphthaleïn as indicator, correspond to 1-2 ccm. of 1/10 normal soda solution. It is advisable to take the amphoteric reaction of milk into consideration, otherwise the peptonised milk may be too acid.

If the milk be cloudy it may be cleared by boiling with white of egg and filtering. Gelatin or agar is to be added after the first filtration.

## (2) Preparing Objects.

**Demonstrating the Tubercle Bacillus in Tissues.\***—Dr. G. d'Arrigo and Dr. R. Stampacchia recommend as fixatives of tissues to be examined for tubercle bacilli the two following solutions.

Pyrogallie acid 2 grm., alcohol (95 per cent.) 100 ccm. The solution must be freshly made as occasion requires, and the pieces, having been first washed in water, are immersed therein for four days. The solution is better renewed at the end of two days. After removal the pieces are transferred to 95 per cent. alcohol (changed every 5-6 days) until it is no longer blackened.

The second solution recommended is Hayem's fluid, which is composed of distilled water 100 grm., sodium chloride 0.5 grm., sodium sulphate 2.5 grm., sublimate 0.25 grm. The pieces, which must be small, are left in this solution for 24 hours, and kept in the thermostat at 37°. After removal they are washed in running water for some hours, and then transferred to alcohol to which a little iodine has been added.

The sections are obtained by the paraffin procedure, and are made to adhere to the slide by the distilled water and heat method. The staining solutions recommended are the Ziehl-Neelsen phenol-fuchsin, followed by Gabbet's methylen-blue, the formula for which is, distilled water 100 ccm., sulphuric acid 50 ccm., methylen-blue 2 grm.

It is advisable to allow the phenol-fuchsin solution to act for 20-30 minutes at a temperature of 40°. The preparation should next be washed in a mixture of spirit and water until the stain is no longer given off, after which it is treated with the methylen-blue solution for a few seconds only. The preparation is then washed in water, which is changed until it is no longer stained, and finally mounted in the usual way.

In the case of sputum, especially when this secretion contains few bacilli, it is advised to mix some with 1/3 alcohol and heat the test-tube in a thermostat for 24 hours at 37°, or for three hours at 50°. The mixture should be frequently stirred up in order to allow the spirit to thoroughly penetrate the sputum. Prepared in this way, sputum will keep for a long time, and bacilli can be demonstrated therein after months and even years.

**Demonstrating the Structure of Coli and Typhoid Bacilli.†**—Dr. A. Wagner has demonstrated the structure of coli and typhoid bacteria by the following method. In 100 grm. of a boiling 1/4 per cent. salt

\* Centralbl. Bakt. u. Par., 1<sup>o</sup> Abt., xxiii. (1898) pp. 64-7, 123-31.

† Tom. cit., pp. 433-6, 489-92 (2 pls.).

solution, 2 grm. of primulin are dissolved. The solution is filtered and allowed to cool. A cover-glass preparation is floated on the primulin solution in a watch-glass, the latter being placed in water at about 60°, and allowed to remain there for some hours, or indeed all night. The preparation is then washed with water, and stained with Hessian Bordeaux for 1/2–2 minutes. The Bordeaux solution is made by dissolving 1 per cent. of the pigment in boiling water and filtering once or twice. The preparation, after having been washed, is mounted.

The cultures from which the preparations were made were from glycerin-agar media, the coli being 20–24 hours old and the typhoid about 13 hours.

**Examining the Nephrostomes of Selachia.\***—M. F. Guitel describes a simple procedure for showing the nephrostomes of the kidney of the adult *Acanthias vulgaris*. The body is opened along the ventral middle line, and Flemming's fluid poured in. The solution, which is composed of chromic acid 1 per cent. 15 parts, osmic acid 2 per cent. 4 parts, and glacial acetic acid 1 part, is allowed to act for a minute and a half. The fixative is then poured off, and the ventral cavity quickly and thoroughly washed with water. As the tissue of the nephrostomes fixes the osmic acid more strongly than the surrounding parts, these organs, stained black to chestnut brown, show up well against the dark grey of the adjacent tissues. The reaction attains its maximum in 24 hours. The pieces thus prepared keep well in alcohol or in 2 per thousand carbolic acid.

**Microscopical Examination of Viscous Urine.†**—Herr G. Michel employs the following method in order to separate the organised deposit in albuminous viscous urines for microscopical examination. 50 ccm. of the urine are shaken several times with 20 ccm. of ether in a cylinder of 100 ccm. capacity and the mixture set aside for some time. The ethereal layer will then contain all the organised elements. It is drawn off with a pipette into watch-glasses, and after evaporation of the ether, the residue is removed to slides for microscopical examination.

**New Method of Decalcifying.‡**—Dr. E. Rousseau has devised the following method for decalcifying.

The objects to be decalcified, which should not be too large, are imbedded in celloidin in the ordinary way, i.e. after fixation, are dehydrated in alcohol, and then, after having been immersed in a mixture of equal parts of ether and alcohol, are impregnated with celloidin in solutions of increasing strength (4 per cent., 8 per cent., 12 per cent.). When the objects are sufficiently saturated with celloidin, the latter is hardened by slow evaporation, by alcohol or by chloroform. The celloidin blocks containing the objects are next immersed in a mixture of alcohol at 90° and nitric acid, the proportion of the latter being regulated by the amount of calcareous matter to be got rid of. The author uses 10 to 50 parts HNO<sub>3</sub> to 100 of alcohol. The decalcifying fluid should be renewed from time to time, and when decalcification is complete, the excess of acid is removed by washing in water and immersion in 90 p. c. alcohol,

\* Arch. Zool. Expér. et Gén., v. (1897) pp. 385–8 (1 pl.).

† Chem. Zeit., xxi. p. 316. See Pharmaceut. Journ., 1898, p. 324.

‡ Bull. Soc. Belge de Microscopie, xxiii. (1896–97) pp. 159–65.

frequently changed, for several days. To the alcohol, carbonate of lime may be added if necessary. When all the acid has been removed, the celloidin block may be sectioned. Now it may happen that, owing to extensive decalcification, there will be large gaps in the objects, which would be a source of considerable inconvenience in making and manipulating the sections. This difficulty is, however, easily got over by filling up the holes with 4 per cent. celloidin, and allowing this to set by evaporation.

This method possesses many advantages; there is no deformity of the animals or tissues, which are observed *in situ*, and it is easy and rapid.

(3) Cutting, including Imbedding and Microtomes.

**New Microtome (System Beck-Becker)\***—Dr. Arno Beck describes an instrument which is intended to imitate the drawing and pressing motions of hand-cutting, and to improve on the pressing motion of most microtome knives.

FIG. 61.

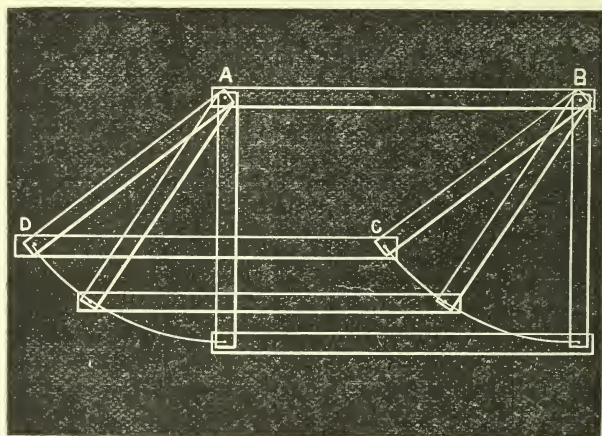


Fig. 61 shows the principle of his knife. A parallelogram of metal rods is jointed at the four corners, and the back edge AB is clamped. DC represents the knife-edge, which, as it is moved forward, imitates the two motions desired. A uniform wear and tear of the knife-edge also results. Regulation of the lengths of the arms also regulates the thickness of the sections; for the more elongated the curve described by the knife-edge, the finer the section. Adjustable screws through A and B determine the pitch of the knife. The guide arms AD and CB are strongly made, and are kept properly placed by strong springs with the intention of preventing the hopping of the knife. A small type of the machine successfully cuts sections of 5 by 7 to 6 by 8 cm.

**New Microtome by Reichert.†**—Dr. J. Nowak, of Cracow, describes this instrument, which resembles the Fromme rocking microtome pre-

\* Zeitschr. f. wiss. Mikr., xiv. (1898) pp. 324-31 (5 figs.).

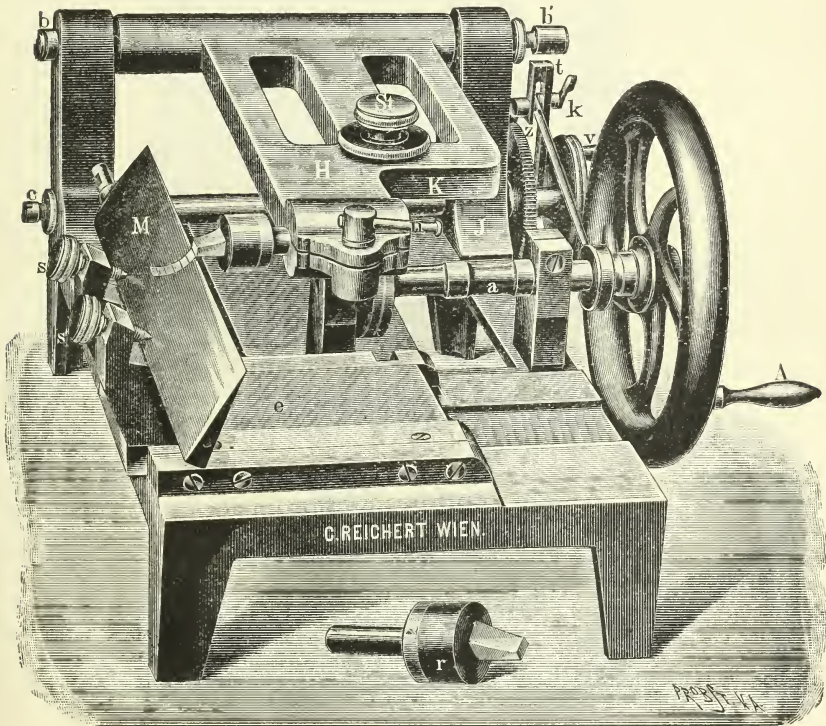
† Tom. cit., pp. 317-24 (3 figs.).



viously described by Schaffer in the same journal,\* but possesses a certain modification in the regulation of the movement apparatus by automatic stop and release mechanism, as well as an apparatus for square sections of the paraffin block. These modifications virtually amount to a new instrument, which seems highly ingenious. The view from above is shown in fig. 62, and from below in fig. 63.

A strong cast-iron base (A in fig. 63) bears on its upper side two upright pillars about 5 cm. high. In the pillars lies a horizontal axis on which a frame H (fig. 62) rotates. The frame carries a screw *St*

FIG. 62.



whose end extends beyond the lower surface of the frame, and passes further on into the groove of an excentric mounted beneath the frame. This excentric wheel runs on an axis *a* mounted above the cast-iron base, which axis carries a winch wheel A of 10 cm. diameter on the end extending beyond the frame.

By the rotation of the large winch, and the consequent rotation of the excentric, the frame H is raised and depressed with a kind of pendulum motion. A corresponding movement follows of the object-holder fastened to its end, and therewith of the object to be cut. The object-

\* Cf. this Journal, 1896, p. 572.

holder is so fixed that the cutting-plane of the object takes a vertical position.

FIG. 63.

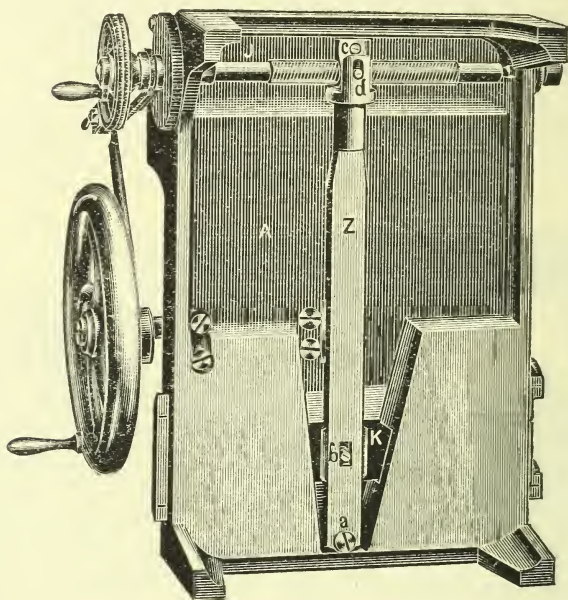
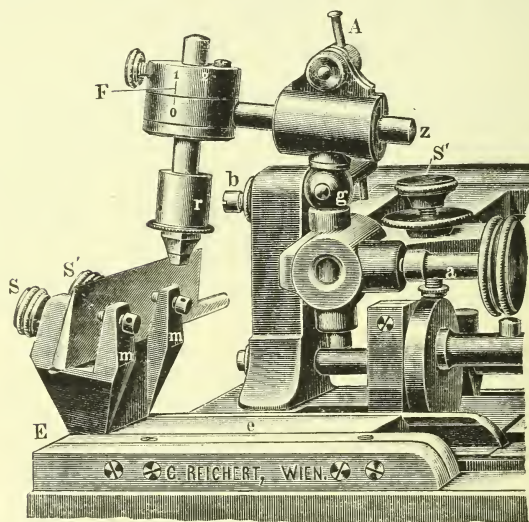


FIG. 64.



The knife moves slowly from left to right without altering its setting; the smaller the knife movement, the thinner the section. The

knife is mechanically connected with the winch, whose rotation produces on the one hand a sec-saw motion of the paraffin block, and on the other a pushing of the knife. This is accomplished by means of a slide (*e* in fig. 62; *K* in fig. 63) about 4 by 15 cm., moving in a groove of the cast-iron base. This slide lies exactly under the object-holder, and carries the knife-holder *ss'* at one end. At the further edge of the base is the micrometer screw *J* (fig. 63), which by means of a three-armed lever is connected with the slider *e* (*K*).

The lever *Z* is under the base *A*, and is at one end connected with a nut *c*, which is fastened to the micrometer-screw at *d*; at the other end there is a small slot *b*, in which a pivot juts out from the knife-slide *K*. The lever is fastened to the base at *a* by a pin around which it can turn. Thus is formed a three-armed lever with its fulcrum at *a*. When the micrometer (endless) screw *J* is turned the nut *c* is displaced, a partial rotation of the whole lever round *a* follows, and a corresponding displacement of the slider *K* is the result. As the arm *ab* is shorter than *ad*, the displacements of *K* are much less than those of the nut *c*. Thus great nicety of working is ensured.

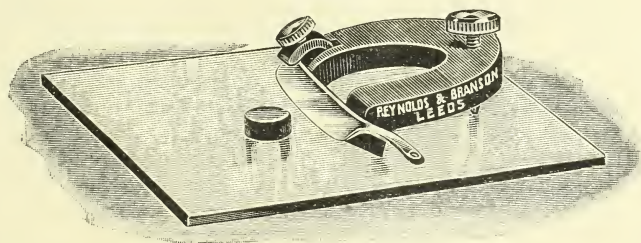
There is a rackwork for adjusting the movements of the knife, whose inclination is regulated by four screws.

Fig. 64 shows an additional piece of apparatus *F*, by which the smallest paraffin blocks can be arranged for section cutting. The arrangement permits the use of those parts of the knife-edge which are not generally in play, and thereby affords something like uniform wear of the blade.

Dr. Nowak speaks highly of the precision of the machine, and of the ribbon bands of sections.

**Student's Microtome.**—Messrs Reynolds and Branson, of Leeds, have made a simple form of microtome, devised by Prof. de Burgh Birch, which will be found very useful to students in physiology, botany,<sup>†</sup> &c.

FIG. 65.



<sup>†</sup>The instrument (fig. 65) is arranged to slide on a glass plate; the substance to be cut is imbedded, and fixed on the glass plate. Sections of any degree of thickness may be cut by raising or lowering the screw, and the microtome is arranged so that any razor may be clamped to it.

Price, with glass plate, without razor .. .. 3s. 9d. each.

Ditto, with roughened plate to secure better attachment of the imbedded substance .. 4s. ..

Razors . . . . . 1s. and 2s. ..



**Two very simple Microtomes.\***—Mr. E. Pinnock describes two very convenient and cheap forms of microtome. (1) The “handy” has a V-shaped groove for the paraffin imbedded or the naturally hard object, the latter being moved forward by a finely cut screw, the object being held in place by the thumb. There is a flat expansion at the end for the razor, which should preferably be ground flat. (See fig. 66.)

FIG. 66.

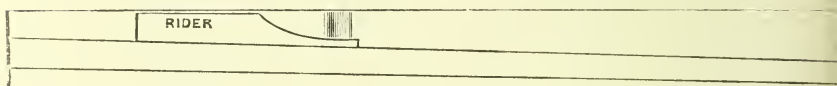
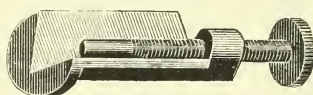


FIG. 67.

(2) Dr. Wetherill's application of the well-known rivet principle to a hand-microtome. It is made of hard wood, with a horizontal portion as a guide for the sweep of the razor, and an incline upon which slides the rider or object-carrier, to which the paraffin imbedded object is attached by melting. In the case of a naturally firm object, the rider may be dispensed with. The object is moved up the incline by the thumb of the hand holding the microtome. (See fig. 67.)

#### (4) Staining and Injecting..

**Contrast-Staining of Bacteria.†**—Herr Knaak has devised a method in which the cells are stained with fuchsin, and the bacteria with methylen-blue. After the action of the methylen-blue, the preparation is decolorised with hydric sulphide solution (1-10). By this procedure the pigment is not removed, but merely reduced, and much less so in the bacteria than in the cells and nuclei. In order to prevent reoxidation of the leucomethylen-blue by the atmospheric oxygen, the preparation is treated with saturated tartaric acid solution. It is then contrast-stained with fuchsin (1 part saturated alcoholic solution to 20 parts water) for 5-10 seconds. Instead of sulphuretted hydrogen solution, 1 per cent. solution of argonin may be used for decolorising, but this must be allowed to act for 4 minutes.

**Simple Method for Staining Spores.‡**—Dr. A. Aujesky has devised the following procedure for staining spores. The cover-glass film, dried in the air, is placed for 3-4 minutes in almost boiling 1/2 per cent. hydrochloric acid. On removal, the cover-glass is washed in water, dried, and then stained with Ziehl's fuchsin solution, after the manner of staining tubercle bacilli in sputum. That is to say, some fuchsin

\* Trans. Amer. Micr. Soc., xix. (1897) p. 189 (2 figs.).

† Deutsch. med. Wochenschr., 1897, No. 42. See Centralbl. Bakt. u. Par., 1<sup>te</sup> Abt., xxiii. (1898) p. 343.

Centralbl. Bakt. u. Par., xxiii. (1898) pp. 329-31.

solution is dropped on the cover-slip, and the latter heated over a flame until it begins to vaporise. This should be done twice.

The preparation having been allowed to cool for 1-2 minutes, is decolorised in 4-5 per cent. sulphuric acid, and contrast-stained for 1-2 minutes in malachite-green or methylen-blue.

Instead of Ziehl's solution, anilin-oil fuchsin or gentian-violet may be used. In this latter case, Bismarck-brown or vesuvin should be used as contrast stain.

**Dahlia as a Stain for Bacteria in Celloidin Sections.\***—Mr. R. C. Reed recommends dahlia for staining bacteria in sections prepared by the celloidin method. The formula used is, saturated alcoholic solution of dahlia 20 ccm., distilled water 100 ccm. The sections are over-stained (15-30 minutes), and then thoroughly washed with 95 per cent. alcohol, until the celloidin around the section appears colourless. They are then cleared up, for which purpose clove oil is preferred.

This method is not, of course, suitable for bacteria which require special stains or treatment.

**Importance of Testing the Reaction of Sputum in Staining for Tubercle Bacilli.†**—Dr. N. G. Ward points out the importance of testing the reaction of sputum, and if it be found acid, to render it alkaline before staining it. Any alkali may be used. The reason given by D. B. Kyle, who was the first to call attention to this fact, is that the capsule of the bacillus is permeable by an alkali, but not by an acid. In staining the sediment of urine, pus, or any secretion or fluid for tubercle bacilli, it is equally necessary to make sure it is alkaline before staining.

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

**Gelatin as a Fixative.‡**—Mr. H. H. Dixon has recently used as a fixative a dilute solution of gelatin in an aqueous solution of bichromate of potash. The solution should be quite fluid at 10° C. The ribbon of paraffin sections is laid on a drop of this solution on the slide. Wrinkles are removed by gently warming the slide. The superfluous fluid is then removed by means of blotting paper, and the gelatin allowed to dry and harden. During this process it should be exposed to a bright light, the action of the light rendering the fixative quite insoluble even in warm water, and so removing all danger of detachment from the slide.

The bichromate has the further advantage of preventing the gelatin, after exposure to the light, from taking up the dyes used as stains.

#### (6) Miscellaneous.

**Paste for Labels.§**—A paste for sticking labels on glass, porcelain, and metal, may be made of gummi arab. 15·0 parts, tragacanth pulv. 7·5, glycerini 45·0, thymoli 0·3, alcohol 3·75, water up to 120·0. The gum arabic is dissolved in 15 parts water, and the tragacanth rubbed up

\* Trans. Amer. Micr. Soc., xix. (1897) pp. 182-5.

† Microscopical Bulletin, xv. (1898) pp. 1-2.

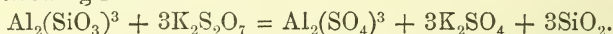
‡ Ann. of Bot., xii. (1898) pp. 117-8.

§ Photog. Zeitung. See Zeitschr. f. angew. Mikr., iii. (1898) p. 364.

with 30 parts water. The two fluids are then mixed and strained. The glycerin is next added, and finally the thymol dissolved in alcohol.

**Test for the Production of Indol by Bacteria.\***—Dr. Th. Smith claims that dextrose-free bouillon is the best medium for testing the production of indol by bacteria. It is prepared from beef infusion, by extracting either in the cold or at 60° C., and inoculated the same evening with a culture of an acid-producing bacterium, such as *B. coli*, and placed in the thermostat. Next morning the infusion is boiled, filtered, pepton and salt added, and then neutralised and sterilised.

**Method for Splitting up Argillaceous Silicates containing Diatoms.†**—Herr G. Marpmann communicates a method for obtaining diatoms from schist and argillaceous earths. It consists in treating the earths or clay with potassium pyrosulphate. The chemical reaction is represented in the following formula.



In this way are formed easily soluble sulphates of aluminium and potassium, while the silicic acid, for the most part in condition of quartz or diatom shells, is not affected, though some of it remains in a soluble state.

In practice the procedure is as follows.

One part of the broken up clay or earth is heated with three parts of the salt, and when melted, one or two parts more of the salt are added. When thoroughly melted, the mass is poured out into a vessel filled with water, and then boiled for a time. If the mass do not thoroughly dissolve, the sediment should be treated with strong hydrochloric acid, and then the diatoms separated by fractional sedimentation.

The salt (pyrosulphate) may be made by heating 87 parts of neutral sulphate of potash in a platinum dish with 49 parts of pure sulphuric acid. When thoroughly melted, the mass should be poured out on to a dry porcelain plate, and, when cold, broken up and preserved in stoppered bottles.

**Making Sections of Steel.‡**—Mr. F. S. Rice prepares sections of steel for the purpose of demonstrating the microstructural characteristics. The pieces should 3/4 in. in diam. Sections should be 3/16 in. thick, if they are to be heated after cutting in the lathe. The best sections are obtained by carefully grinding off the surface to a plane by hand on an ordinary quick-cutting oil-stone, then on the finest Belgian oil-hone, and finally polishing on a piece of chamois tightly stretched over a block of wood and charged with peroxide of tin. When thoroughly polished, the surface is washed with alcohol followed by a little chloroform. The polished surface is then etched in order to develop the structure.

Though this can be done by means of nitric acid and water, the surest and most delicate results are obtained from a saturated solution of iodine in alcohol diluted with an equal bulk of alcohol, both 95 per cent. Several applications of the iodine solution may be required. After each etching, wash in 95 per cent. alcohol, and dry quickly to

\* Journ. Expér. Méd., ii. (1897) pp. 543-7.

† Zeitschr. f. angew. Mikr., iii. (1898) pp. 341-5.

‡ Trans. Amer. Micr. Soc., xix. (1897) pp. 28-42 (3 pls.).



prevent oxidation. Then polish with chamois, mount on a slide without a cover-glass, and examine.

A thin coating of vaselin is the best protection from oxidation.

**Old Book on Optics.**—The following figures (68–71) from Zahn's 'Oculus Artificialis' (1702), to which Mr. E. M. Nelson referred at the meeting in November last,\* have been made from photographs kindly taken for the purpose by Mr. C. L. Curties.

FIG. 68.

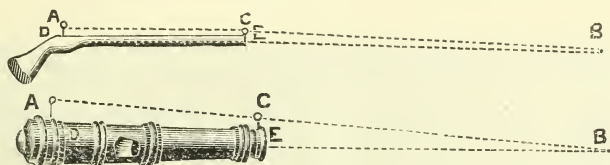


Fig. 68 illustrates a telescope-sight for a musket and a cannon. Zahn says, "Bombardæ et omni generi balistarum ac tormentorum bellicorum tubum opticum sive telescopium aptare, quo visus ad scopum exactè dirigi poterit."

FIG. 69.

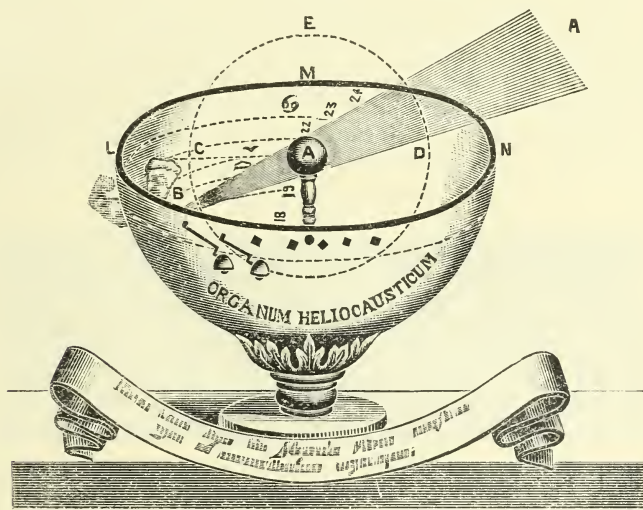


Fig. 69 is the Jordan sunshine recorder. The legend in the ribbon below is

"Horas Luce Sono tibi sphærule Vitrea monstrat,  
ignis nil mirum Coelicus urget opus."

The first thing that attracts attention is the converging nature of the sun's rays incident on the sphere! Next, it will be observed that

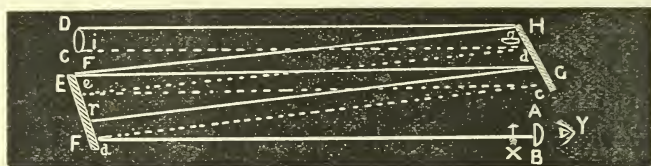
\* Journ. R.M.S., 1897, p. 600.

in the figure the principal focus of the glass sphere is about 8 instead of one and a half radii, as it ought to be. An instrument constructed on the proportions as shown in this figure would therefore be useless. This is a strange mistake, because the focus of a sphere is correctly given in another part of the book.

It is intended that at the hours the sun's rays should burn a string to which a bell is attached, thus causing it to ring.

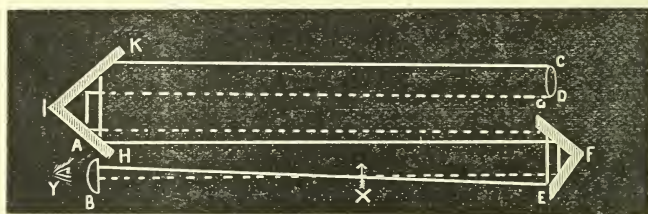
We now come to the most interesting figures, viz. 70 and 71. These are called "*Catoptrico dioptrica telescopica*," and are two out of a series

FIG. 70.



of seven. In fig. 70, DC is the object-glass of a telescope, and AB its eye-lens; by means of the two mirrors HG and EF the telescope is, as it were, folded up into five. The arrow at X is intended to represent an erect image. It will be noticed that there are two reflections at each mirror. This is the second in the series; the first has not been copied, as the arrangement can easily be understood from fig. 70; the difference between them being that there is only one reflection at each mirror instead of two, so that the telescope is folded into three instead of into five. This leads to fig. 71, which is the third of the series, and shows an arrangement of plane mirrors, by which the path of the rays is diverted

FIG. 71.



in precisely the same manner as by Porro's prisms. This is the principle which underlies the Zeiss patent binoculars. It will be noticed that the eye-lenses in figs. 70 and 71 are plano-convex, and are turned in their proper positions.

The seventh and last of the series has four plane mirrors, and is very ingenious.

The axis of the telescope is supposed to be horizontal; the rays are intercepted just behind the object-glass by a plane mirror placed at an angle of  $45^\circ$ , and are reflected vertically downwards on to the second plane mirror, placed nearly horizontally. This reflects the rays verti-

cally upwards on to the third mirror, also placed horizontally, which reflects them again downwards on to the fourth mirror, placed at an angle of  $4^{\circ}$ , and this turns them finally into a horizontal direction to the eye-piece.

A telescope of 10 ft. focus thus constructed would, when its axis is horizontal, have a vertical height of about  $4\frac{1}{2}$  ft., the distance between the eye-lens and the object-glass being about 1 ft. The object-glass and the eye-lens are in a line with one another, and are placed in the middle of the telescope.



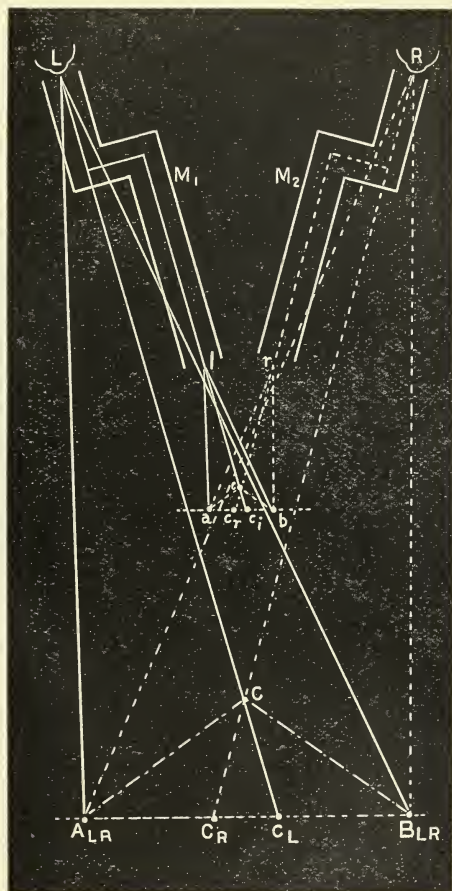
## MICROSCOPY.

## A. Instruments, Accessories, &amp;c.\*

## (1) Stands.

Greenough's Stereoscopic Microscope and its Auxiliary Appliances.†—Herren S. Czapski and W. Gebhardt, of Jena, commence their

FIG. 75.



article by setting forth the advantages of a binocular Microscope, and by discussing the conditions which should be aimed at in the construc-

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

† Zeitschr. f. wiss. Mikr., xiv. pp. 289-312 (7 figs.).

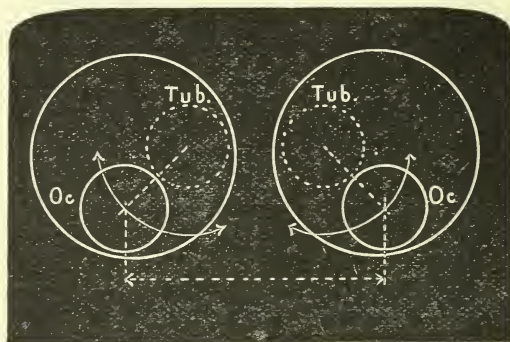
tion of such an instrument. A sketch of the history of Greenough's endeavour to solve the problem is also given. In analysing the conditions of orthomorphic vision, Greenough's equation is arrived at, viz.

$V = \frac{D}{d}$ , where  $V$  = the linear magnification of the single Microscope;

$D$  = distance of the observer's eyes;  $d$  = distance of the light entrances of both Microscopes. Another condition can be thus expressed:—"The image must in all its parts in each Microscope-tube appear from the point of sight under the same angular distance as the object from the focus of the primary rays;" or still more simply, "Entrance-pupils and exit-pupils of the Microscope must be on the same points of junction" (*Knotenpunkte*).

In order to investigate this last condition, one must realise that corporeal images are never seen as such, but construct themselves by an unknown psychic process out of two different plane retinal images. Let us take three points  $a, b, c$  (fig. 75) not lying in a straight line as the

FIG. 76.



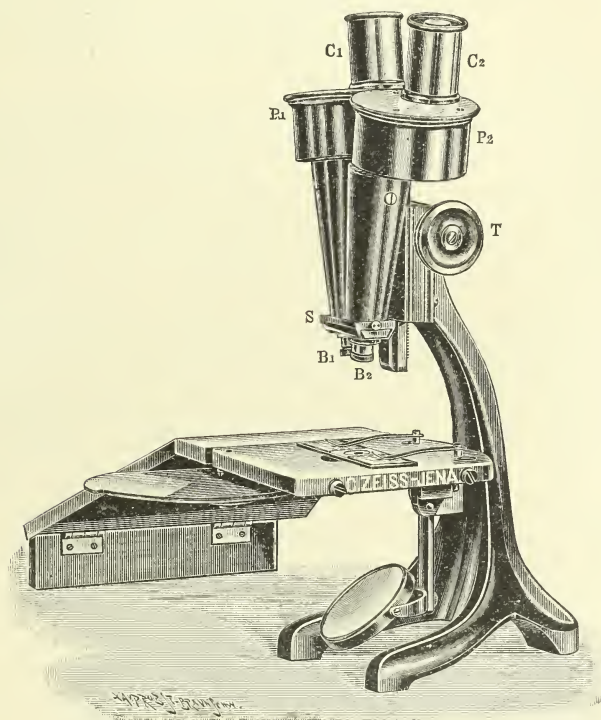
object, and imagine two Microscopes  $M_1, M_2$  inclined to one another at an angle of about  $14^\circ$ , and so directed towards the group of points that the picture of all three falls in the field of view of both Microscopes. Let  $l, r$ , be the "entrance-pupils" of both Microscopes in Abbe's sense, i.e. the focus of the image-forming pencil of rays, and consequently the perspective projection centre for representation. Let  $c_l, c_r$  be the projections of the point  $c$  on the line  $ab$  produced backwards from  $l$  and  $r$ . Then the working of the Microscope confines itself to projecting in all its parts an equally magnified reproduction of  $abc_l$  and  $abc_r$ .

The determination of the visual angles under which the images of  $a, b, c$  and  $a_l, b_l, c_l$ , i.e.  $A_L C_L B_L$  and  $A_R C_R B_R$ , must be presented to both eyes in the case of normal accommodation, requires that the optical axes should virtually intersect in a point  $C$ , whose distances in space from  $A$  and  $B$  stand to one another in the same ratio as those of  $c$  from  $a$  and  $b$ . This is satisfied only when the angles, under which the image-points  $A_L, B_L, C_L, A_R, B_R, C_R$  appear simultaneously to the eye, are equal to those under which the corresponding object-points  $a, b, c$  from  $l$  and  $r$  respectively appear. Increase of this angle would be in stereoscopic

view too great an approach of C to A, B, i.e. a relative flattening of the corporeal image, diminished plastique; reduction of the angle, on the contrary, would effect too great a separation of C from A, B, i.e. exaggerated plastique.

The points of an optical system from which the views of objects indifferently proceed are well known by Listing's name of "junction-points" (*Knotenpunkte*), and they stand to one another in the relation of image and object. Therefore the simplest expression of Greenough's condition for orthomorphy is as given above, viz. "Entrance-pupils, &c."

FIG. 77.



From this the formula  $V = \frac{D}{d}$  can be easily deduced; for  $\frac{D}{d} = \frac{RB}{rb}$  (by similar triangles).

A system of Porro prisms is used; and, in order to avoid the derangement incidental to the lengthening of the tube for adaptation to different eyesights, a rotation of the tube is employed, which causes an eccentric rotation of the oculars, and allows wide limits of adaptability. (See fig. 76.)

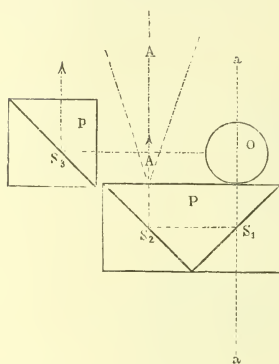
As stops are usually placed near the object, and for many purposes would be a hindrance, they are here adapted for unscrewing. But for the attainment of orthomorphy they are quite inadmissible, as a com-



parison of sight with and without these stops teaches. Instead of the "entrance-pupils," here the lower "junction-points," it is more convenient to apply stops to the "exit-pupils" or upper "junction-points."

The objective lenses are brought together on a small slide working sideways with a push motion until the fine-adjustment screw is needed. This slide can eventually be fitted with stronger or weaker objectives.

FIG. 78.



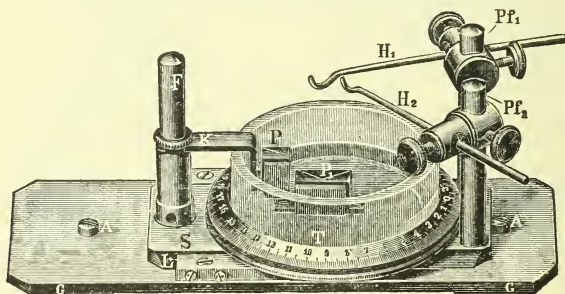
One of the objectives permits of an in-and-out screwing movement, to produce sharp definition to the corresponding eye. Complete presentation to both eyes is an essential condition of perfect stereoscopic vision. Oculars of different strengths can be fitted.

The auxiliary apparatus includes,—(A) a prism rotator, and (B) a capillary tube-stage rotator.

A. The design and working of this little apparatus are easily understood from fig. 78. The object O is placed on the hypotenuse plane of a reflection prism P, so that it is exactly perpendicular over the middle of one of the two silvered right-angled planes  $S_1$ . When the examination of O from above is finished, and the observer wishes to examine the under side, he has merely to push the prism P with the object thereon sideways until O comes under the axis A, and to lower the corresponding tube. A second half-sized prism p, worked also sideways, will give a side view.

The framework containing these prisms, and the mode of adjusting them, is shown in fig. 79, which is fully described in the original paper.

FIG. 79.

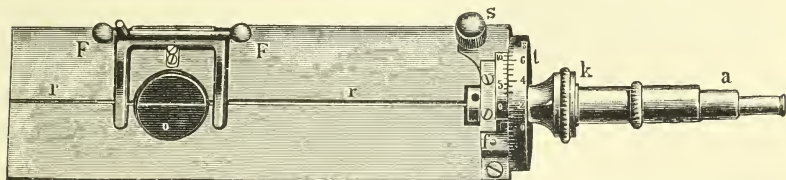


B. The object of this (fig. 80) is to facilitate observation with the highest powers—even with immersion objectives—of minute objects which it is desired to view from all points, but whose examination by an ordinary method of rotation would be tedious, and to which much movement might perhaps be productive of injury.

A rectangular plate bears in the direction of its long axis a triangular channel  $r$ , which passes through a circular sunk opening  $o$ . The cir-

cumference of this orifice has underneath a slight projection bearing a glass plate, the whole forming a chamber of slight depth (about 0.75 mm.). The function of the chamber is to regulate the flow of the homogeneous-immersion medium, so as to avoid injury to the object by

FIG. 80.



alteration of the position of the cover-glass. A screw and drumhead divided into degrees works in an axially perforated cylinder, and effects the rotation of the chamber.

A list of advantages claimed for the apparatus closes the description.

**Two Old Microscopes exhibited by the President at the last Meeting.**—The first (fig. 81), which was made by Benjamin Martin, is an attempt to improve Cuff's "new constructed double Microscope" (1744), with what success we shall presently see. The instrument, which is mounted on a folding tripod in place of the square box foot, differs essentially from Cuff's, as it is a stage focuser. Cuff's Microscope had a sliding coarse adjustment, the limb which carries the body sliding on a square pillar fixed to the box foot. A jamb screw was attached for the purpose of fixing the limb at a mark engraved on the square pillar, corresponding to the number on the objective on the nose-piece. Alterations in focus were then made by the fine-adjustment screw, which caused the body either to approach or recede from the stage.

In Martin's the body remains fixed while the stage is moved up or down by rack and pinion, until the stage is brought to a mark engraved on the fixed pillar, corresponding to a number on the objective. Parallel to the fixed pillar is a square steel bar, to which the stage may be clamped by a jamb screw. When the fine-adjustment screw is turned, it moves this square steel bar up or down, carrying with it the stage, and in doing this, let it be noted, it turns the coarse-adjustment pinion! This is, of course, a much inferior method to that of Cuff's, both on account of the stage focusing, and also with regard to the manner it is effected, which causes a superabundance of friction. The loss of time on the fine-adjustment screw amounts to no less than one and a half revolutions!

There are six objectives, with a single lieberkuhn common to them all; the lieberkuhn slides on a long nose-piece, after the manner of Cuff's, marks being engraved to indicate its proper position with any given objective.

There are also six magnifiers for use as simple Microscopes; three of these are fitted with proper lieberkuhns. There is an ordinary spring slide-holder, as well as a supplementary stage with transverse motion in arc, an arrangement peculiar to Martin's Microscopes. The limb

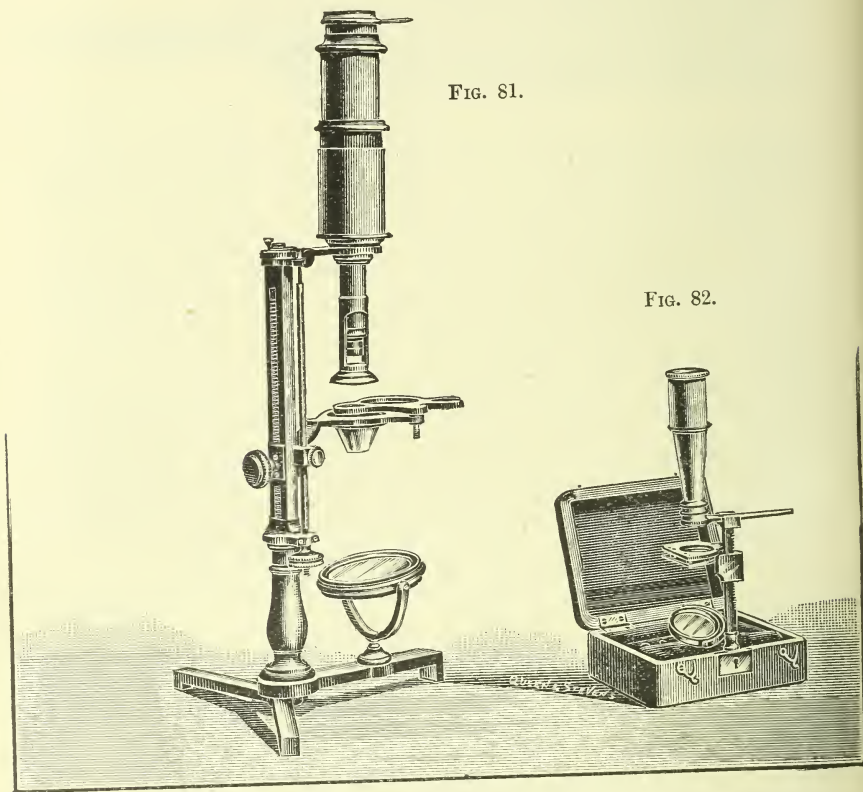
which holds the body is hinged so that the body can be placed in a horizontal position. The limb has also motion in arc with a pin to fix it in the optic axis.

There are two concave mirrors, one of 4 in. and the other of 9 in. focus.

The optical portion of this Microscope is peculiar. The eye-lens is a doublet consisting of a crossed lens of  $1\frac{1}{4}$  in. focus and a plano-convex of  $1\frac{3}{4}$  in. focus, placed in their proper positions, their combined focus

FIG. 81.

FIG. 82.



being 1 in.; the field lens is a plano-convex of 3 in. focus, properly placed, the distance between the field lens and the compound eye-lens being  $1\frac{3}{4}$  in.

There is at the upper end of the long nose-piece an equi-convex lens of  $5\frac{1}{2}$  in. focus; this must be considered a part, not of the eye-piece, but of the objective. In reality it is the back lens of the objectives, for it is common to them all; so that, in changing the objectives, it is only the front lens that is changed. This, which, optically speaking, is an improvement, is probably the invention of Martin. The same optical arrangement was employed by Adams in 1771. Opticians copied one



another so freely that, in the absence of any published account, it is difficult to determine either the originator of any particular improvement or the date of its introduction. As, however, Benjamin Martin was a mathematician, who understood both the theory and practice of optics, this invention may be safely credited to him. The body has a draw-tube.

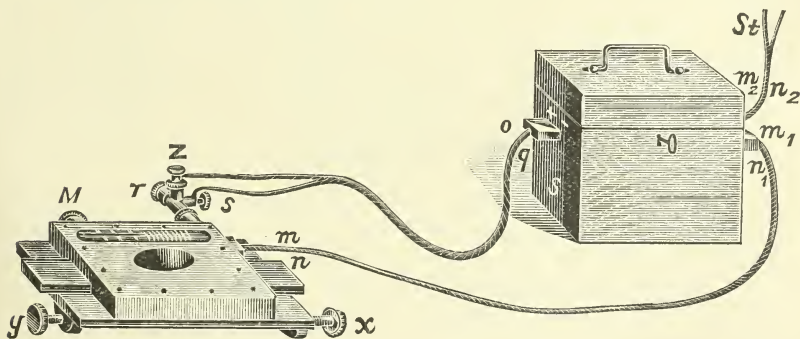
The date of this Microscope must be placed between 1759 when Martin came to London, and 1782 when he died. If, therefore, we say between 1760 and 1770, we shall not be far wrong. It is very portable, the outside measure of its box being  $10\frac{1}{2} \times 7\frac{1}{4} \times 2\frac{1}{2}$  in., and its weight in box with apparatus  $4\frac{3}{4}$  lbs.

The second Microscope (fig. 82) is by Cary; it is a small simple Microscope of a common form, fitted with rack-and-pinion stage focussing. The vertical pillar screws into a brass plate, let into the box where the hasp is usually placed.

It has also a compound body which may be used in conjunction with the simple lenses. This compound body, which is only  $2\frac{3}{4}$  in. long, has a compound eye-lens, similar in arrangement to that of Martin's described above. There are four powers, which screw into one another in the usual manner. The box measures only  $3\frac{3}{4} \times 3 \times 1\frac{1}{4}$  in.

**Electrically Heated and Regulated Warm Stage.\*** — Dr. Rudolf Kraus, after summarising the various heating stages that have been invented since the first (by Stricker in 1871), points out that no new application of electric methods has taken place since Stein's in 1884. Stein's method was to place in the hollow stage a spiral connected with

FIG. 83.



an electric current; but this plan had the faults of deficient regulation and liability to great variations of temperature, due to the fact that air is not a good conductor of heat, and therefore is not adapted for a steady constant heating medium.

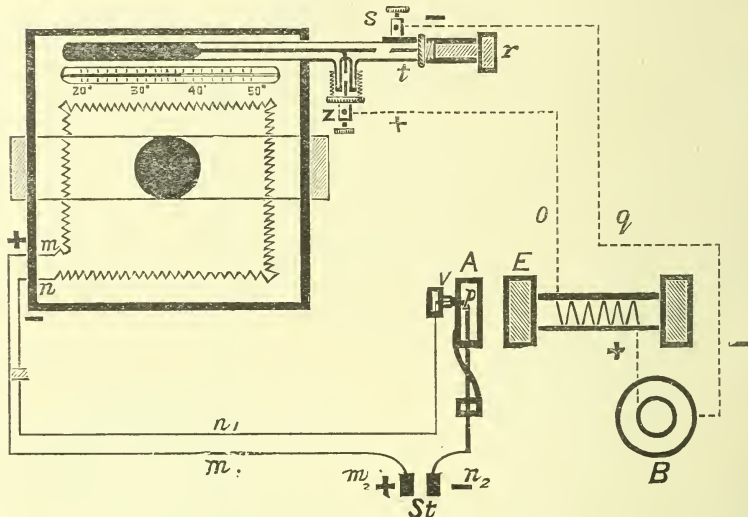
The next idea was to make use of liquids as conducting media; and after many attempts Herr Ehman has succeeded in finding one, viz. paraffin oil, which conducts heat and is not itself decomposed by the current.

\* Centralbl. Bakt. u. Par., xxiii. (1898) pp. 16-20 (2 figs.).

The electrically heated object-stage consists of the stage proper, silver spirals, paraffin oil, an ordinary graduated thermometer, and a contact thermometer. There is also a relay (a Neefhammer). The object-stage proper is a deep metal box containing a silver spiral connected with the main current by  $m_2 n_2$ . The regulating apparatus consists of a contact thermometer inserted in the stage and the relay in a wooden box. The contact thermometer is connected with the relay, and closes and opens the current going to the relay from the battery B.\*

The apparatus is worked as follows:—Contact  $m_2 n_2$  is made with  $St$  the main current, and the current entering by  $m n$  into the spiral heats it. The paraffin thus becomes warmed, and the temperature continues to rise until the quicksilver expanding reaches the point of the

FIG. 84.



platinum thread  $t$ . At this moment the current  $o q$  of the element B is closed and the hammer A from E. Contact is opened by  $v p$ , which, in the rest position of the relay, is shut; and the main current  $m n$ ,  $m_2 n_2$  is opened. No more heat now goes through the spiral, and the temperature in the stage sinks. Therefore, when the quicksilver thread of the contact thermometer operates, the adjoining current  $Z S O Q$  is opened and the main current again introduced. The alteration of the currents is marked by an audible click from the hammer. Only a very weak current (0.2 ampère) goes through the spiral. It was found possible to regulate the temperature to  $0.1^\circ$ , and to keep it constant even for a whole day.

\* The contact thermometer is apparently in the liquid, and only its extremity is visible. This arrangement would allow of the conduction to the liquid of any heat which would be generated in the contact thermometer, so tending to counter-balance the loss by radiation and keep the paraffin at a constant temperature.—Ed.

In addition to this advantage of sure regulation and constancy of temperature, the object-stage admits of a rapid attainment of a desired temperature as well as of a rapid alteration.

The attainment of a desired temperature (e.g.  $37^{\circ}$ ) occurs during the passage of the main current. When the thermometer shows  $37^{\circ}$ , then the platinum thread is brought into contact with the quicksilver thread, and a distinct click is heard from the relay. The temperature then remains constant, and the main current, being shut off, is replaced by the auxiliary current.

A higher or lower temperature is easily attained. To get a higher temperature, e.g.  $45^{\circ}$ , the platinum thread is withdrawn to a proper distance by rotating the screw-head until the graduated thermometer shows  $45^{\circ}$ . The quicksilver and the platinum threads in the contact thermometer meet; the main current is shut off and the relay called into action as before. A reverse movement of the platinum thread combined with exclusion of the currents permits a cooling down somewhat below the desired temperature. Then by manipulation of the currents the temperature is raised to the desired point.

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

**Zeiss' New Comparison Spectroscope.\***—This instrument is intended to assist botanists and physiologists in the study of such coloured substances as chlorophyll, and is so named because its special feature is to render possible an exact comparison of the absorption spectra of solutions. As will be seen from the accompanying figure (fig. 85), the apparatus has the general form of a Microscope, and so far resembles one that the special spectral arrangement can be applied to a Microscope stand. The object-table F bears two orifices, 4 cm. apart, through which two mirrors reflect the sun or lamplight perpendicularly upwards. Each of these two pencils, passing through prisms situated in the horizontal box, becomes decomposed and forms a spectrum. The two spectra appear in close proximity, and can be observed through the broad slit C in the eye-piece.

In the side tube D is placed the wave-length scale, which is illuminated by the mirror at the tube's mouth, its image being projected between the two spectra. The width of the slit, and consequently the brightness of the spectra, can be altered by the turning of the knob A.

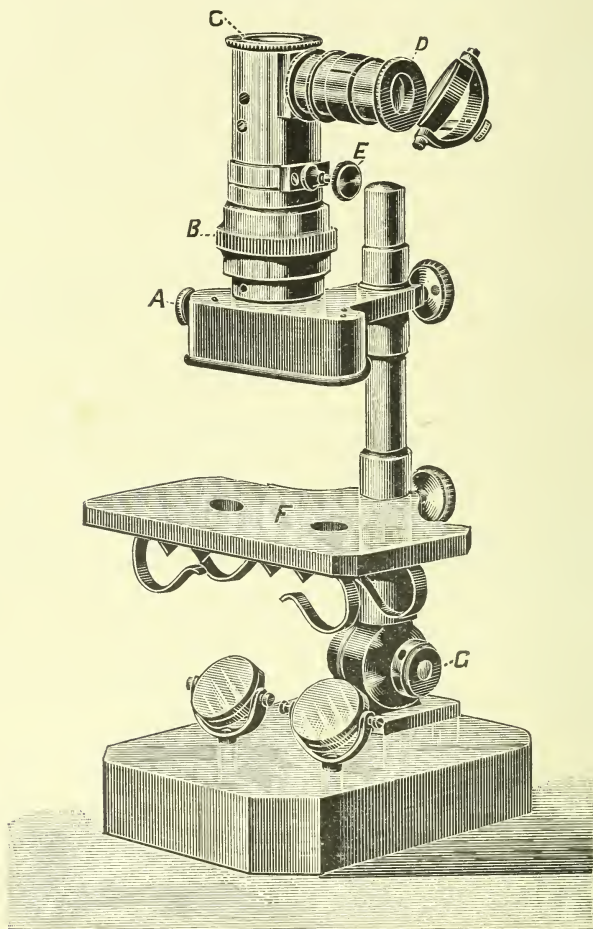
Rotation of the tube-piece B brings out sharp presentation of the scale and spectra; the screw E causes a side movement of the scale image. The two plane and concave mirrors are movable round vertical and horizontal axes. When the necessary mirror adjustments have been made, two spectra with the most important of Fraunhofer's lines are seen over one another. It is easy by movement of the scale to get the D line on  $a = 589$ . When daylight is not available, a spirit-lamp with a salted wick or asbestos thread soaked in NaCl solution may be used. When direct sunlight is used, the number of Fraunhofer's lines that appear is endless. The cross-piece carrying the prisms and superincumbent ocular slides up and down in a vertical axis, and can be clamped at any desired height.

\* Bot. Centralbl., lxxiii. No. 10, pp. 349-52 (1 fig.).



For comparative observation of absorption spectra of various solutions it is best to use Zeiss' double absorption vessels. The manage-

FIG. 85.



ment of the instrument is easy and remarkably convenient, and its great feature is its adaptability with even relatively unfavourable light.

(6) Miscellaneous.

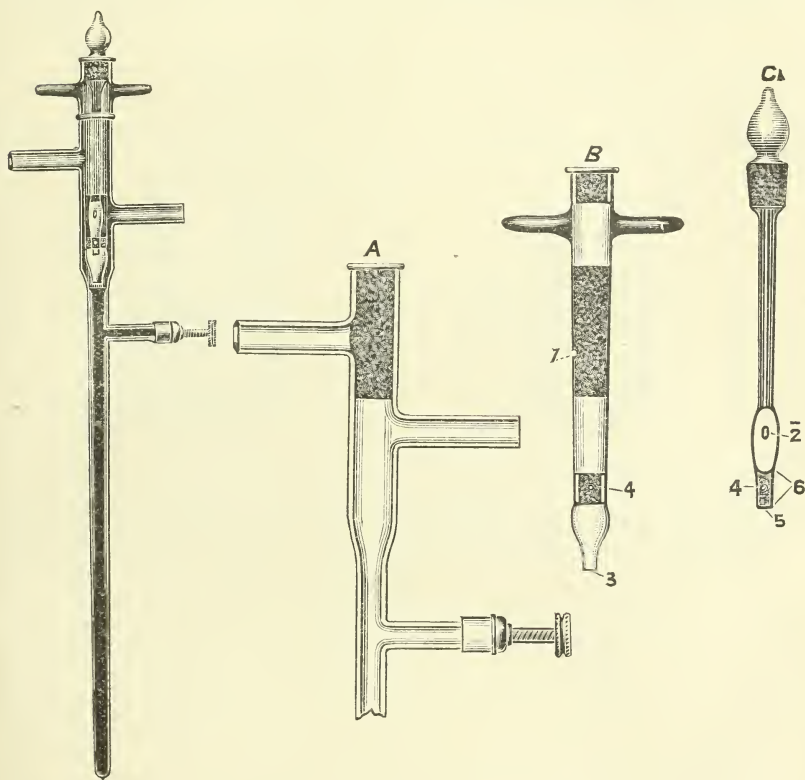
**New Thermo-Regulator.\***—Dr. F. G. Novy has devised a thermo-regulator which can be used for high as well as low temperatures. Fig.

\* Journ. Applied Microscopy, i. (1893) pp. 91-2 (2 figs.).

86 shows the apparatus as a whole, reduced in size. Fig. 87 illustrates the construction of the regulator. Part A is provided with two lateral tubes which have an internal diameter of 6 mm., and has a ground internal surface indicated by the stippled portion of the drawing. Part B is ground to fit the preceding. It is provided with an opening (1) through which the gas passes into the interior. The lower portion of B has a smaller opening (4) 1 mm. in diameter. The lower portion of tube B has an internal diameter of 2 mm., and when placed in position

FIG. 86.

FIG. 87.



inside of A, it should come within one or two millimetres of the bottom of the cup. In this case, the first droplet of mercury, as it issues from below, shuts off the outflow of gas. The upper part of C is solid, and the lower part hollow. The gas enters through a large opening (2) and passes down to (4) the minimum outflow, and to (5) and out at (3). The portion marked (6) is ground to fit exactly the corresponding part in B. If this is not done properly, the minimum outflow of gas cannot be regulated as perfectly as it can be otherwise. The upper portion of C is a ground stopper fitting into B. The manipulation is simple. The gas

enters through the upper lateral tube. If it is desired to diminish the inflow of gas, this can be done by turning B. The gas passes through (1) into the inner space and leaves at (2). The gas goes down, and a portion leaves through the minimum supply opening (4), while the remainder passes down through (5) and out of (3). By turning C the minimum outflow of gas can be regulated at will. The regulator works exceedingly well, especially in connection with Murrill's gas pressure regulator.

**New Gas-Pressure Regulator.\***—Mr. P. Murrill has invented a gas-pressure regulator, the strong points of which are efficiency, simplicity, durability, and cheapness. The outer vessel or pail is 6 in. (15 cm.) in

FIG. 88.

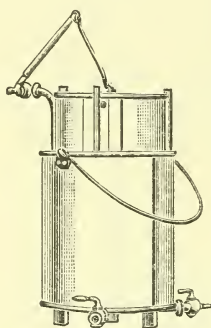
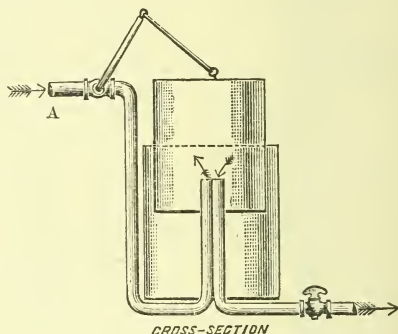
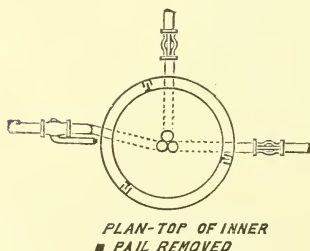


FIG. 89.



diameter and 7 in. (18 cm.) deep. Through the centre of the bottom three tubes enter, rising  $5\frac{3}{4}$  in. (14.5 cm.) above the bottom. The direction and arrangement of these tubes is shown in the illustrations.

FIG. 90.



The inlet tube (fig. 89, A) is fitted with a stopcock to which a 4-in. (10 cm.) lever arm is attached. On the inside of the pail are soldered three vertical U-shaped grooves extending 3 in. (8 cm.) above the top. The inner vessel or float is 5 in. (13 cm.) in diameter and 6 in. (15 cm.) deep. On the outside are soldered three vertical flanges, corresponding to the three grooves in the outer vessel. To the top of the float is soldered a stiff wire ring, and this is connected with the lever arm by a wire of such length that the valve is wide

open when the float is at its lowest position. The float with attachments should weigh about 25 oz. (700 gm.), under which weight the gas will be delivered at about 40 mm. pressure; but by means of weights placed

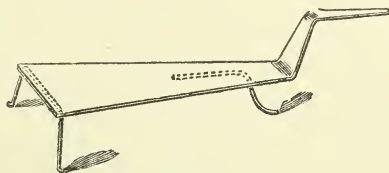
\* Journ. Applied Microscopy, i. (1898) pp. 92-4 (4 figs.).

on the float the pressure at which the gas is delivered may be varied at will. The outer vessel is to be filled with water to the depth of 5 in. (13 cm.); or glycerin or liquid paraffin may be used.

The operation of the apparatus is as follows. One of the two exit tubes may be connected with a manometer, or both may go to burners. Gas enters through the stopcock into the float which, as it rises, closes the valve. If the outlets be closed, the float will rise until the valve is entirely closed, in which position it will stand. When the exit tubes are opened the float falls, the valve reopens, and gas is admitted at the same rate at which it is consumed. The apparatus, which is made of metal, is designed to be used in connection with a thermostat, and with it the temperature may be held constant within  $0.1^{\circ}\text{C}$ .

**Paraffin Imbedding Table.\***—Mr. H. B. Ward has devised a modification of the ordinary paraffin imbedding table, which is superior to the older type in several respects. The table is made of a triangle of sheet copper with a base of 6 in. and a perpendicular height of 14 in.

FIG. 91.



The edges of the triangle are turned under and inward, giving to the table a slightly rounded margin. In height, the main part of the table measures 2 in., and it is about 4 in. high under the apex of the triangle where the flame is applied. The legs are made of five-sixteenths copper rod bent as shown in the illustration, and riveted to the copper sheet.

**Circular Colonometers.**—Mr. H. W. Jeffers† has constructed an apparatus (fig. 92) for counting the colonies of bacteria on circular plates. It consists of concentric zones which are divided into sections, each having an area of 1 sq. cm. The Petri dish can be centred upon this apparatus by the circles, and the area read from the line its edges approach. To facilitate the reading of the area of the plate, the circles 80 and 120, whose areas are equal to 80 and 120 sq. cm. respectively, were drawn as dotted circles, thus making the areas, marked *a* and *b*, equal to one-half a square centimetre. The colonies in several areas can be counted, an average taken, and the result multiplied by the number of square centimetres in each plate.

The colonometer devised by Mr. J. Weiss‡ (fig. 93) is made up of eight concentric circles and 92 sector circles. The first or centre circle is 1 cm. in diameter; the second 3 cm.; the third 5 cm.; the fourth 7 cm., and so on to the eighth, which is 15 cm.

\* Journ. Applied Microscopy, i. (1898) pp. 88-9.

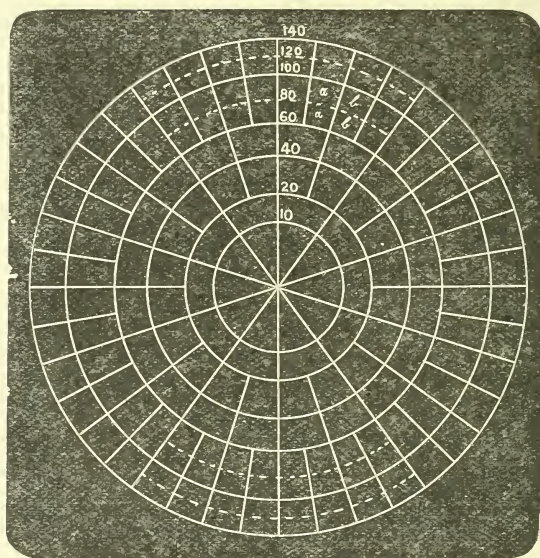
† Tom. cit., pp. 53-4 (1 fig.).

‡ Tom. cit., pp. 54-5 (1 fig.).



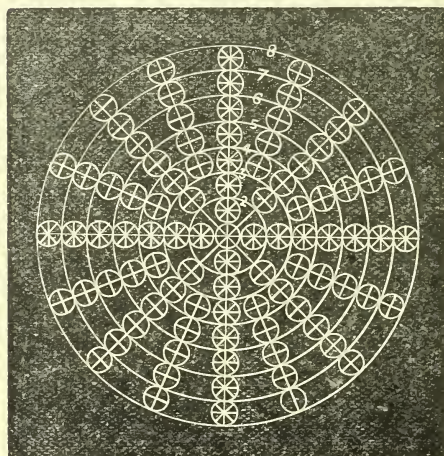
The counting is done as follows. An average number of colonies is found in one of the sector circles, in a definite working area, and the number is multiplied by the ratio of the area of the sector circle to the

FIG. 92.



Diameter one-half actual size.

FIG. 93.



Diameter one-third actual size.

area of the entire plate. The ratios are  $2:1 = 9$ ;  $3:1 = 25$ ;  $4:1 = 49$ ;  $5:1 = 81$ ;  $6:1 = 121$ ;  $7:1 = 169$ ;  $8:1 = 225$ .

Thus, if in a sector circle of circle 6, the average number of colonies were 12, then the number of colonies is  $12 \times 121 = 1452$ .

**New Test-Plate.\***—Dr. H. van Heurck, after alluding to the scarcity of Nobert's plates, suggests that microscopists may avail themselves of the markings of diatoms for the purpose of testing the resolving power of lenses. The author has carefully worked out the following table, giving in parallel columns the diatom-equivalent of Nobert's series.

Nobert's series stops at 19, but the author has continued the calculation for 20, 21, and 22.

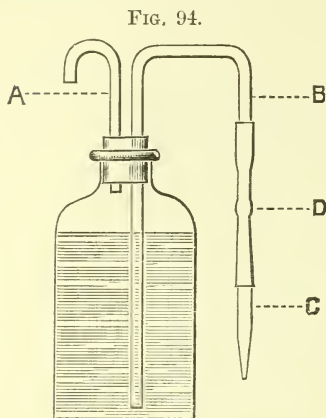
Nobert's Series.	Number of Lines.	Number of Diatom Lines.	Corresponding Diatoms.
1	443	445	<i>Navicula dactylus</i> (str. trans.)
2	665	620	„ <i>lyra</i> „
3	886	900	<i>Epithemia turgida</i> „
4	1108	1100	<i>Navicula Brebissonii</i> „
5	1329	1330	<i>Stauroneis phoenicenteron</i> (str. trans.)
6	1550	1500	<i>Pleurosigma balticum</i> „
7	1572	1800	„ <i>angulatum</i> „
8	1994	2000	<i>Mastogloia meleagris</i> „
9	2215	2200	<i>Surirella gemma</i> „
10	2437	2400	„ „ (str. long.)
11	2658	2600	<i>Mastogloia exigua</i> Lew. (str. trans.)
12	2880	2800	<i>Van Heurckia rhomboides</i> typ. (str. trans.)
13	3100	3100	<i>Nitzschia linearis</i> Sm. (str. trans.)
14	3323	..	„ „ var. <i>tenuis</i> (str. trans.)
15	3544	3400	<i>Frustulia saxonica</i> (str. trans.)
16	3766	3600	„ „ (str. long.)
17	3987	$\left\{ \begin{array}{l} 3800 \\ \text{to} \\ 4000 \end{array} \right\}$	<i>Amphipleura pellucida</i> (str. trans.)
18	4209	4200	„ „ small (str. trans.)
19	4430		
20	4652		
21	4872		
22	5093	5000	„ „ (str. long.)

**Improved Form of Wash-bottle.†**—Mr. W. C. Sturgis describes a wash-bottle devised by Mr. A. L. Winton. The apparatus is easily made, easily manipulated, and under perfect control. It consists of a glass

\* Zeitschr. f. angew. Mikr., iv. (1898) pp. 1-4.

† Journ. Applied Microscopy, i. (1898) pp. 75-6 (1 fig.).

vessel, the neck of which is plugged with a rubber stopper with two perforations, through which pass two U-shaped tubes. To one of these is fitted a glass nozzle through the intermediary of a piece of rubber tubing. Into the rubber tube is pressed a solid glass ball with a diameter larger than that of the lumen of the rubber tube. The vessel having been filled, the flow is secured by merely pressing the tube immediately above the head D. In this way a steady stream or single drops are made to issue from the nozzle (fig. 94).



**The Microscope and its Application.\***—The concluding portion of Prof. L. Dippel's work on the Microscope and its Application has recently appeared. It contains the third and fourth sections which deal with the structure of the stem, the root and

leaves, and also with the development of the various parts of plants. It is copiously illustrated with woodcuts.

### B. Technique.†

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

**Method for Making Anaerobic Roll-Cultures with Gelatin or Agar.‡**—The following simple method for making anaerobic roll-cultures is given by Herr G. Marpmann. Two test-tubes are required, one of which can be pushed just inside the other. The larger is then 1/4 filled with the medium and sterilised. The medium is inoculated, and then stirred up with a glass rod for 5–10 minutes. The smaller tube is now jammed inside the larger, its surface having been previously sterilised in the flame. The layer of inoculated medium is set by plunging the tubes into cold water. The ends of both are sealed with paraffin or covered with a rubber cap. The colonies are easily counted. Inoculations can be made from any particular colony by merely withdrawing the inner tube, or by cutting through the glass.

**Cultivation Media suitable for Tropical Climates.§**—M. A. Morel, writing from Java, where the mean temperature is over 25° C., states that he has been in the habit of using for some time past a culture medium which remains firm at the hottest season of the year. It is composed of 12½ parts white gelatin, 0·25 parts agar, and 90 parts

\* Braunschweig, F. Vieweg u. Sohn, 1898, pp. 445–644, with Index, Contents, and 132 woodcuts.

† 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.

‡ Zeitschr. f. angew. Mikr., iv. (1898) pp. 37–8.

§ Tom. cit., pp. 4–6.

bouillon. This mass filters well through a hot-water filter, it is only liquefied over  $30^{\circ}\text{C}$ ., and makes excellent plates which set in half an hour without the aid of ice.

The addition of the small amount of agar does not prevent the liquefaction of the medium by peptonising bacteria, though the action is less rapid than on gelatin alone.

The gelatin may be clarified in the usual way by means of egg albumen or by means of "clearing clay," a magnesium-aluminium hydrosilicate, which in conjunction with water is decomposed into the hydrate; and this in the nascent condition has the property of throwing down all muddy and even colouring matters, leaving a clear and colourless supernatant fluid. When the gelatin or agar solutions are ready for filtering, 1 per cent. of clearing clay is added, and having been well shaken the mixture is steamed for one hour before it is filtered.

**Production of Plague Serum.\***—Herr G. Gabritschewsky, finding that living cultures of plague bacillus were unsuitable for immunising horses, made experiments for the purpose of obtaining immunising and toxic substances from cultures of *Bacillus pestis* by plasmolysis. In bouillon with 4 per cent. glycerin the plague bacillus grows freely, on pure glycerin at  $37^{\circ}$  not at all. Horses were immunised by means of cultures sterilised by the addition of glycerin. Agar plates were inoculated from bouillon cultures, and in 24–48 hours at  $37^{\circ}$  a copious plague culture was obtained. This was distributed in test-tubes, each containing 2 cm. of glycerin. In 24 hours a cloudy slimy mass was formed, and this diluted with an equal bulk of bouillon was injected subcutaneously into horses. At the injection site considerable infiltration occurred, and the temperature rose to  $39.2^{\circ}$ . On intravenous injection the temperature rose to  $40^{\circ}$ , falling again after 24–48 hours. This immunising method is only suitable for horses, as glycerin by itself is poisonous to mice, and in rabbits and guinea-pigs causes local necrosis.

The rest of the paper is devoted to the plasmolytic method of sterilising cultures.

**Culture of Pleurococcus.†**—Miss Dorothea F. M. Pertz recommends for the culture of this organism Knop's solution 0.2 per cent. in sterilised glass dishes and flasks; also in hanging drops of the same solution. Chodat's filamentous form of the alga did not occur.

## (2) Preparing Objects.

**Method for Preparing Plankton Organisms.‡**—Herr G. Marpmann gives the following for preparing soft organisms such as plasmodes, Radiolaria, Infusoria, &c. A 1 per cent. solution of hydrochlorate of cocain is added to the fluid to be examined in the proportion of one-third to one-half the volume of the latter. Observation under the Microscope shows that the organisms, even the lower plants, lie quite still, their processes, tentacles, &c. being fully extended. A drop of formalin is then added. This kills and fixes the organisms at once.

\* Russ. Arch. f. Pathol., klin. Méd. u. Bakt., 1897. See Centralbl. Bakt. u. Par., 1<sup>te</sup> Abt., xxiii. (1898) pp. 808–9.

† Rep. Brit. Ass. Toronto, 1897 (1898) p. 864.

‡ Zeitschr. f. angew. Mikr., iv. (1898) pp. 42–3.



It is now a very easy task to stain such objects, and mount them in glycerin, glycerin-jelly, or balsam. If they are mounted in balsam the preparations should be successively treated with alcohol, xylol, and lavender oil. Care must be taken lest the organisms get dry during the process.

**Fixing and Preparing Freshwater Algæ.\***—F. Pfeiffer R. v. Wellheim recommends the following process. The fixing fluid is composed of equal parts in volume of 40 per cent. formol, pyroligneous acetic acid, and methyl-alcohol. After decanting the greater part of the water from the alga, the fixing fluid is added, and must be at least double in volume of the water that remains. The algæ may remain in this fluid for weeks or months. If the alga is of a gelatinous character, it must be transferred from the fixing fluid into strong alcohol; and this must always be done before examining or staining.

Algæ fixed in this way may be stained by any of the familiar colouring reagents. Very favourable colouring solutions are the following:—(1) 100 ccm. of 50 per cent. alcohol, with 2–3 ccm. of iron chloride solution in 90 per cent. or in absolute alcohol; (2) A concentrated solution of perfectly pure carmin-acid in 50 per cent. alcohol. Before staining the object must lie in 50 per cent. alcohol. It must then remain in solution (1) for at least 4–6 hours, and be then freed from the excess of iron chloride by 50 per cent. alcohol. A few drops of solution (2) are then added; and the staining is completely effected in a few hours. The object is next placed in 95 per cent. alcohol, and then transferred to the imbedding substance.

By this process the structure of *Spirotaenia trabeculata*, and the honey-comb structure of *Oscillatoria princeps*, are very well shown.

**Preparing Parasitic Fungi.†**—Prof. A. N. Berlese adopts the following method of preparing microscopic fungi parasitic on leaves. The special advantage is that the observer can at once detect whether the fungus is in the stage desired for examination, without waiting the result of a long and tedious process.

Pieces of the leaf infected by the parasite are placed in absolute, or in 96 per cent. alcohol, or in concentrated picric acid, or better, in a mixture of 15 ccm. 0·5 per cent. chromic acid, 6 ccm. 1 per cent. osmic acid, and 1 ccm. glacial acetic acid, and then in concentrated nitric acid. After washing in distilled water and boiling in alcohol, they can be examined under a low magnifying power. For imbedding, the material is hardened in absolute alcohol and then placed in chloroform in a closed glass vessel. The pieces of infected leaf remain floating on the chloroform. Alcohol is then run in until the material is completely covered by it. After a short time the two fluids mix; the material becomes impregnated, and falls to the bottom of the vessel. The saturation is generally completed after from four to six hours, and the pieces of leaf are then placed in a concentrated solution of paraffin in chloroform or directly in pure paraffin melting at 50° C. After sectioning, the sections are freed from paraffin by turpentine-oil; then placed in absolute alcohol until required for staining.

\* Oesterr. Bot. Zeitschr., xlviii. (1898) pp. 53–9, 99–105.

† Jahrb. f. wiss. Bot. (Pfeiffer u. Strasburger), xxxi. (1897) pp. 166–70.

**Simple Method for Demonstrating the Production of Gas by Bacteria.\***—Mr. H. E. Durham describes a modification of the fermentation tube which consists in placing a small test-tube in an inverted position within the ordinary test-tube used for cultivations. The small tube should be freely movable when placed inside. The tubes are filled by means of a burette, and are then steam sterilised on three successive days. In most cases the smaller tube becomes completely filled after the first sterilisation, and it is not usual for a bubble to persist after the second. The tubes must not be tilted too much when they are inoculated (fig. 95).

FIG. 95.



**Demonstrating Pacini's Corpuscles.†**—Pacini's corpuscles, which may be regarded as the highest development of nerve-endings, are easily demonstrable by *intra vitam* staining. The animal is to be injected with a saturated solution of methylen-blue at 37°, and when dead the pieces are to be stained in saturated aqueous solution of picrate of ammonia. Methylen-blue forms with picrate of ammonia a combination insoluble in water, but soluble in spirit. The preparations are then immersed in a solution of molybdate of ammonia 1, water 20, aceto-hydrochloric acid 1 drop. With molybdic acid, methylen-blue forms a compound insoluble in alcohol, and now the preparations can be dehydrated, imbedded, cut, stained, and mounted without detriment.

**Formol-Methylen-Blue Treatment of Nerve-fibres.‡**—Herren G. Rossolino and W. Muraview adopt the following procedure for the demonstration of normal or diseased nerve-fibres. The pieces are placed for one or two days in 2-2.5 per cent. formalin, and then transferred finally to 4 per cent. formalin. After at least four days the formalin may be exchanged for 95 per cent. alcohol, and the pieces teased out or sectioned. The pieces or sections are stained in a hot 0.5 per cent. aqueous methylen-blue solution. After the solution has cooled down, the preparations are transferred to 1 per cent. alcoholic solution of anilin for a few seconds or for some minutes. They are then washed in 95 per cent. alcohol to remove the anilin, cleared up in cajeput oil, and mounted in balsam.

By this procedure two different kinds of nerve-fibres can be distinguished. In one of these the medullary sheath is studded all over with small round granules of variable size and form. The method is satisfactory also for the nerve-cells and the connective tissue elements. The preparations are permanent, retaining the stain well, but they do not bear exposure to sunlight.

**Demonstrating the Nucleoli of Cells in Central Nervous System.§**—Herr V. Růžička reports a method for demonstrating the histo-

\* Brit. Med. Journ., 1898, i. p. 1387 (1 fig.).

† Zeitschr. f. angew. Mikr., iv. (1898) pp. 38-41.

‡ Neurol. Centralbl., xvi. (1897) pp. 722-7.

§ Zeitschr. f. wiss. Mikr., xiv. (1898) p. 452-5.

logical structure and peculiarities of nucleoli in the cells of the central nervous system. Pieces of spinal cord, 0·5 centimetre thick, may be hardened in alcohol, or they may be first fixed in saturated solution of sublimate, or in commercial formol diluted to one-half, and then after-hardened in alcohol.

The celloidin sections are immersed for 10 seconds in warm 1 per cent. aqueous methylen-blue solution, or in carbol fuchsin. The sections are decolorised in alcohol, and then placed in chloroform, which dissolves out the celloidin. After dehydrating in alcohol and clearing in cajeput oil, the sections are mounted in Canada balsam.

By this method, while the nuclear mass remains unstained, the nucleoli or nuclear corpuscles are highly coloured, and show one centrally placed body, or several extremely small granules with no definite distribution and a crenated appearance.

Bowhill's solution \* is also recommended as a good stain for demonstrating the nucleoli.

**Peroxide of Hydrogen in Microscopical Research.**†—Herr R. Volk recommends peroxide of hydrogen for killing motile and sensitive animals of minute size. One drop of three per cent. solution to 2 ccm. of water suffices for some Rotatoria, and 1 drop to 1 ccm. of water kills Anuræ and other Loricata. The solutions should always be as weak as possible, especially as delicate species are damaged by the oxidising action of the stronger mixtures. When the animals are dead, the mixture must be replaced by water, or by water with 0·3 per cent. of salt. When washed the animals are fixed in the usual way. For objects which contain carbonate of lime the peroxide must be perfectly free from acid.

**Preparing Permanent Blood-Films.**‡—Herr A. Zielina prepared blood-films by receiving a minute drop of blood on one side of a cover-glass, and then distributing it over the surface by touching it with the edge of a slide which is drawn across the cover-glass from left to right. A clean edge is necessary for each film, and the wound should be wiped before taking the next drop.

The films are dried in the air, and are fixed by drawing them eight or nine times through the flame. When cool they are stained for 30 seconds in Nicolle's one-third eosin (saturated alcoholic solution of eosin 50 ccm., 95 per cent. alcohol 100 ccm.). The superfluous stain is removed, and the preparation dried. The film is again stained in ripe Ehrlich hæmatoxylin for 30–40 minutes. The cover-glass, film side downwards, is then suspended on water for a time. It is again stained with the one-third eosin solution for 30 seconds. After having been washed with water, it is dried, and mounted in balsam.

**How to examine the Blood and Diagnose its Diseases.**§—Dr. A. C. Coles has, by producing a compact account of methods for examining the

\* See this Journal, *ante*, p. 244.

† Zool. Anzeig., xix. (1896) pp. 294–5. See Zeitschr. f. wiss. Mikr., xiv. (1898) p. 469.

‡ Zeitschr. f. wiss. Mikr., xiv. (1898) pp. 463–4.

§ London, Churchills, 1898, 260 pp. and 6 pls.

blood and for the diagnosis of its diseases, conferred a benefit on those who are desirous of becoming practically acquainted with the technique of blood preparations and with the pathology of the blood. The work is divided into three parts, which deal with methods for examining the blood, the general morphology of the blood, and the diseases in which the blood is primarily or secondarily affected.

The author does not lay claim to originality, modestly avowing that the work is more or less a compilation. It is obvious, however, even from a superficial examination, that he speaks authoritatively on a large number of points, and that the knowledge conveyed is the result of personal experience and acquaintance with the subject. The coloured illustrations are extremely effective.

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

**Combination of the Paraffin and Celloidin Methods of Imbedding.**\*—Mr. U. Dahlgren adopts the following method for imbedding ova and embryos of amphibia. The objects are infiltrated with celloidin in the usual way, and then immersed in a large quantity of chloroform for twenty-four hours. They are then transferred to a bath of one-half chloroform and one-half cedar oil. In twenty-four hours they are placed in the water-bath in paraffin of the grade that will be finally used to imbed them. Several changes are necessary, and more time must be allowed than for tissues imbedded by the plain paraffin method.

### (4) Staining and Injecting.

**Action of Pigments on Living Sponges.**†—Dr. G. Loisel finds that granules of Congo-red and tournesol-blue are absorbed by endodermic and mesodermic cells of *Reniera ingalli* and *Spongilla fluviatilis*. Various kinds of colouring matter in solution (safranin, iodine-green, &c.) are stopped at the surface of the sponge, while others again are readily absorbed. Congo-red and tournesol-blue are peculiarly changed inside the cells enclosing them, as if by the action of an acid. After a time, the substances are got rid of into the intercellular spaces, and are taken up by phagocytes, or transported outwards by the contractions of the matrical substance. Loisel goes on to notice the colouring of some living Protozoa, Medusæ, and other animals by various reagents.

**Staining Blood-Films.**‡—Dr. H. Rubinstein recommends the following procedure for staining blood. The cover-glasses should be very thin and perfectly clean. All traces of grease must be removed by passing them several times through the flame. The drop of blood placed on the cover-glass should not be larger than a pin's head. The second cover-glass should be superimposed so that the two form an octagonal figure; and, when the blood has spread out, the two are to be separated without the one exerting any pressure on the other.

After having been dried in the air, the films are fixed by heating the cover-glasses film side downwards on a copper plate about 30 cm. long

\* Journ. Applied Microscopy, i. (1898) p. 97.

† Journ. Anat. Physiol., xxxiv. pp. 187-234 (1 pl. and 3 figs.).

‡ Zeitschr. f. wiss. Mikr., xiv. (1898) pp. 456-62.



and 9 cm. broad, the heat being applied at one end of the plate by a gas or spirit lamp.

The part of the plate to be used for fixation is the area lying between the zones where water boils and where it assumes the spheroidal state. The time required is from half to one minute. The films are stained with Ehrlich's triple stain for 5, 6, or 7 minutes. After having been washed with water, they are dried and mounted in balsam.

**Staining the Envelope of Milk-Globules.\***—Mr. W. Narramore states that by staining milk with eosin and with picrocarmin, he found that the structure surrounding the fat-globules became stained, and that without doubt the much debated membrane of the fat-globule was present. At the same time, this was not universally the case, as a large number showed no trace of a covering. The author remarks that Storch, a Danish investigator, has also succeeded by means of picrocarmin in staining the suspected envelope, which is more condensed or viscous towards its inner side, and more watery on its outer side.

Under the Microscope the fat-globules, colourless in themselves, are seen to be surrounded by a narrow faint red border.

**Staining the "Vacuole-Granules" in Yeast-Cells.†**—Herr E. Küster states that the "vacuole-granules" of yeast-cells, by immersing a sample of cake-yeast in a thin aqueous solution of neutral red, 1 to 5000 or 1 to 10,000, are sufficiently stained in a few minutes. The granules are stained dark red, the rest of the cell remaining uncoloured. If the action of the stain be prolonged, the vacuoles, by absorbing more and more of the pigment, eventually become non-transparent, and look like dark red balls lying in the colourless plasma.

**Double-Stain for Gums.‡**—M. L. Lutz recommends the following formulæ respectively for (1) a hydro-alcoholic solution of red extract of Cassella, and (2) a hydro-alcoholic solution of acid green J E E E (Poirrier):—(1) Rouge de Cassella 0·25 grm., 90 per cent. alcohol 20 grm., distilled water 30 grm.; (2) Acid green J E E E (Poirrier) 0·10 grm., 90 per cent. alcohol 20 grm., distilled water 30 grm. He uses them for the double staining of sections of roots or root-stocks containing gums.

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

**Method of Preserving Culture Media.§**—Mr. F. T. Bioletti preserves culture media by means of the following simple device, which merely consists in the use of a second plug of antiseptic cotton. The test-tubes are cleaned and plugged in the ordinary way, except that the plug is only half the usual length. After having been sterilised and filled, the plug is pushed in to half an inch below the top of the tube, and a plug of antiseptic cotton is put over it. The tubes are sterilised in the usual way, and may then be covered with a rubber cap or be put in a closed vessel to prevent evaporation. The antiseptic cotton is

\* Rep. Liverpool Micr. Soc., 1898, pp. 23-4.

† Biol. Centralbl., xviii. (1898) p. 306.

‡ Bot. Gazette, xxv. (1898) p. 280.

§ Journ. Applied Microscopy, i. (1898) pp. 72-3.

prepared by soaking ordinary cotton in a solution of water 100, alcohol 20, copper sulphate 3. The cotton should be dried slowly, as it chars easily.

By this method tubes can be kept unchanged for at least three months, i.e. there is no evaporation and no contamination.

**Fixative Solutions.\***—Dr. K. Tellesniezky discusses the value of a large number of fixative fluids, and gives a very long list of these solutions and the original references thereto. The solutions are considered in order of ascending complexity, simple solutions of one reagent being first treated of. The double, triple, and quadruple solutions are subdivided into those which do and those which do not contain acetic acid. In fact, the author's paper is in praise of the neglected virtues of acetic acid. This acid, used in connection with plasma-preserving reagents such as potassium bichromate and osmic acid, gives extremely satisfactory results.

There are numerous formulas containing bichromate, osmic acid, and acetic acid, and other fixatives such as sublimate, picric acid, and nitric acid. These are too well known, under the names of their inventors, such as Perenyi, Flemming, Altmann, Rabl, Zenker, to need further description.

To a mixture of 3 grm. potassium bichromate, 5 ccm. acetic acid, and water 100 ccm., the author's own name is attached. Small pieces of material are left in this fluid for one or two days, and larger ones for longer. After removal they are thoroughly washed in water, and then further hardened in alcohol of increasing strength, beginning at 15 per cent.

**Method for the Preservation of Protoplasmic Spinnings.†**—Mr. G. F. Andrews gives the following as being the best method for the preservation of protoplasmic processes. The fumes of 2 per cent. osmic acid are concentrated by heat in a glass chamber. For this was used a large closed tube whose base rested on each side on a glass slide, leaving just space enough in the centre for a third slide to be slipped in and out. The whole stood on a glass plate on a water-bath. The central slide carried a watch-glass of osmic acid solution whose ingress and egress was provided for by a hole in the glass tube, which can be closed by a rubber band. When the fumes were sufficiently concentrated, the central slide was replaced by another of like size. On this the eggs had been previously arranged with just enough water to cover them. After fixation and removal from the chamber, the eggs were washed in many changes of water. The exact length of time the eggs should be exposed to the fumes cannot be given; but in any case it is very short, a second too much or too little either way spoiling the result. The best preservative was found to be concentrated sea-water.

**Method for Fixing Leucocytes and Blood-plates.‡**—Dr. H. Deeljen recommends that the blood drawn from the finger should be received on a film of agar, to which 0.75 per cent. of NaCl has been added. On this the cover-glass is placed. When examined on the warm stage the white corpuscles are seen to be in an actively motile condition. The preparations were next fixed with formalin or osmic acid vapour, and afterwards may be stained in the usual way.

\* Arch. Mikr. Anat. u. Entwickl., lii. (1898) pp. 202-47 (1 pl. and 38 figs.).

† Zeitschr. f. wiss. Mikr., xiv. (1898) pp. 447-52.

‡ München. Med. Wochenschr., 1897, pp. 1192-3.

**Selenium as a Mounting Medium for Diatoms.\***—Selenium, says Herr G. Marpmann, is an element of a brown colour, of sulphur-like consistence, with a refractive index nearly as great as that of sulphur, and when heated melts to a brownish-red fluid. By itself alone, selenium is too dark for a mounting medium, but by mixing with sulphur or arsenic this disadvantage is obviated. By mixing equal parts of *sulfur depuratum* and *selenium metallicum*, rubbing them together and melting in a test-tube, a red fluid is obtained, and this on cooling becomes a bright yellowish-red, and maintains its transparency for a considerable time. It seems that when mounted the preparations are cloudy or even opaque in places, and in order to remove these defects it is advisable to place the mounted preparations in a thermostat at 150°.

Of selenium compounds, selenium ethyl  $\text{Se}(\text{C}_2\text{H}_5)_2$  is a fluid which possesses a boiling point between 107° and 108°. In this fluid considerable quantities of sulphur and selenium can be dissolved. In this way is produced a thick oily fluid, having a refractive index of about 1.90, and forming a medium which should meet the requirements of diatomists.

**Picro-formalin in Cytological Technique.†**—Mr. Graf states that the best fixative for demonstrating the cell-structure of the lower animals is picro-formol in varying strengths, 1 vol. saturated aqueous solution of picric acid and 1 vol. of 5, 10, or 15 per cent. formol, or 95 vols. picric acid solution and 5 vols. formol, or 90 vols. picric acid solution and 10 vols. formol. The animals (*Clepsine nepheloidea* sp. n. Whitm.) are placed (alive) in the mixture for 30 minutes. After immersion in alcohol of increasing strength up to absolute, they are imbedded in paraffin. Sections 3  $\mu$  thick were stained with hæmatoxylin and Bordeaux-red. By this procedure the delicacies of the cell structure, network, microsomes, and vacuoles, are rendered extremely clear.

**Preservative for Freshwater Sponge.‡**—Mr. F. L. Washburn finds the following solution to be of value for preserving freshwater sponges. Pure glycerin 2/3; 3 per cent. formalin 1/3. In this the green sponge will keep for at least three months, the liquid remaining perfectly clear, and allowing the external anatomy of the sponge to be beautifully seen.

#### (6) Miscellaneous.

**Microbiological and Serotherapeutical Technique.§**—M. A. Beson's guide to laboratory work, dealing with the technique of microbiology and serum therapeutics, is one that deserves to obtain universal approbation. It is divided into a general and special portion, the first describing the various methods in use at laboratories, the second describing the numerous species of pathogenic organisms. In a third section the bacteriological examination of water and air is treated of.

\* Zeitschr. f. angew. Mikr., iv. (1898) pp. 6-8.

† State Hospitals Bull., 1897, No. 1; also Neurol. Centralbl., xvi. (1897) p. 550. See Zeitschr. f. wiss. Mikr., xiv. (1898) p. 469.

‡ Journ. Applied Microscopy, i. (1898) p. 73.

§ Paris, 1898, 223 figs. See Zeitschr. f. wiss. Mikr., xiv. (1898) pp. 519-20.

**Camera Drawing.\***—Mr. G. F. Andrews devised a method of camera drawing which proved invaluable for recording delicate appearances. Instead of white drawing paper, he used dark shades of thin dull surface tinted papers, varying the shade and hue according to the amount of light, on the brightest days using black. The point of the pencil was whitened with Chinese white. This device obviated the necessity for using smoked glasses in the camera. There was a notable gain in light and definition. Various coloured crayons to differentiate optical planes or cell-series were used, and sometimes a brass or an ivory point.

\* Zeitschr. f. wiss. Mikr., xiv, (1898) pp. 451-2.

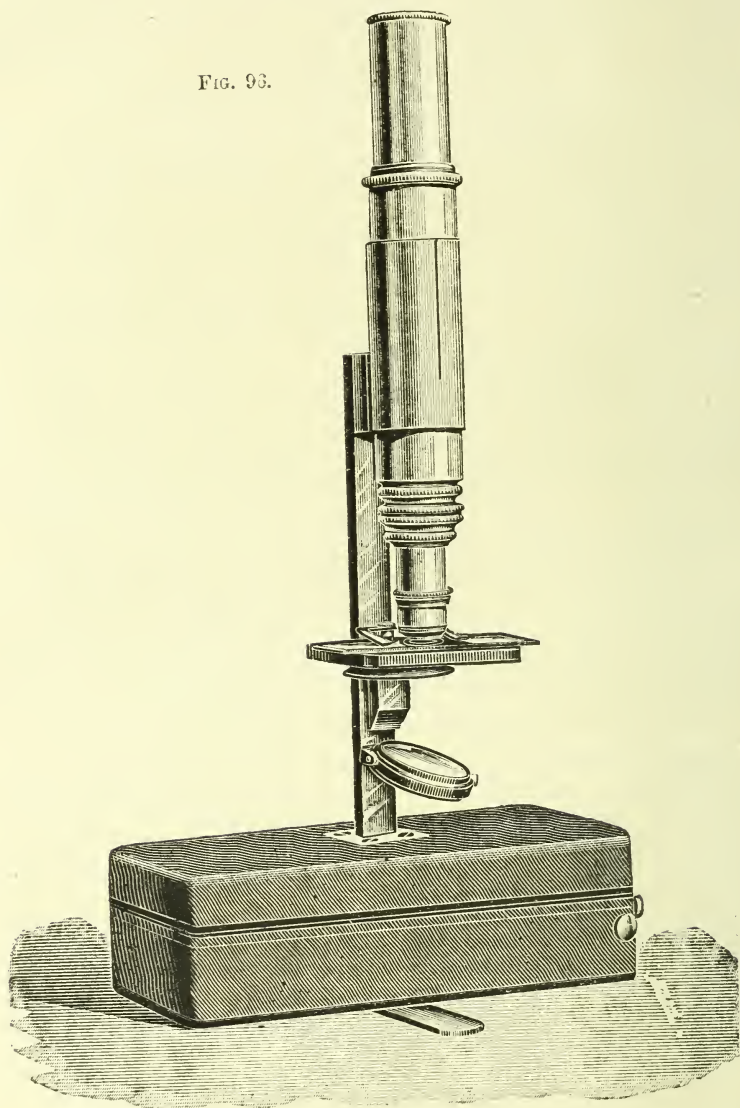


## MICROSCOPY.

## A. Instruments, Accessories, &amp;c.\*

## (1) Stands.

FIG. 93.

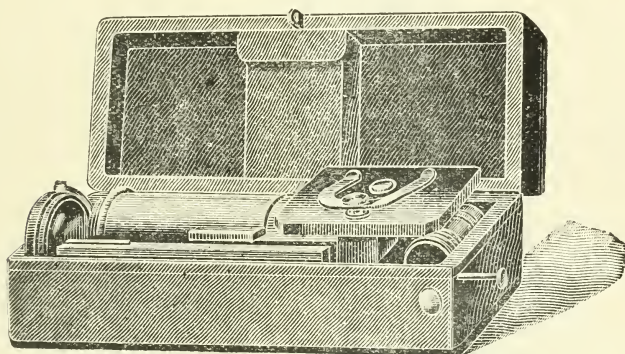


\* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous.

**Sticker's Travelling Microscope.\***—Under this name Dr. George Sticker describes a new form of portable Microscope made by the firm of Leitz to his design.

The general arrangement will be easily understood from the two figures (figs. 96 and 97). When packed in its leather case it does not exceed  $15 \times 5\frac{1}{2} \times 6$  cm. The lid of the case serves as a stand, and a metal rod carries the object-table, mirror, diaphragm, &c. The coarse adjustment is attained by sliding the tube, and the fine by a screw between the tube and the objective. The case contains places for an ocular

FIG. 97.



and two objectives. Only the optical upper part of each objective is packed, and one lower part. The traveller selects the system to suit his purpose. Finer objectives secured in their metal cases can be also packed.

The weight of a waterproof pocket carrying the leather case, and a second case for immersion oil, stain solutions, slides, and cover-glasses, is only 920 grm. (2 lbs.), which weight also includes a leather strap for slinging the whole on one's shoulder like a field-glass.

**Berger's New Microscope.†**—The novelty in this instrument is entirely confined to the overstage, and is intended to meet the constructional difficulties involved in adapting the adjustments (especially the fine) to Microscopes used for high-power photomicrography. Usually the strain on the fine adjustment caused by the extreme length (and consequent weight) of tube is a decided disadvantage. Herr Berger arranges so that both his coarse and fine adjustments are independent of the draw-out. The Microscope is also suitable for ordinary use.

Fig. 98 gives a general view, fig. 99 a vertical section, and fig. 100 a ground plan.

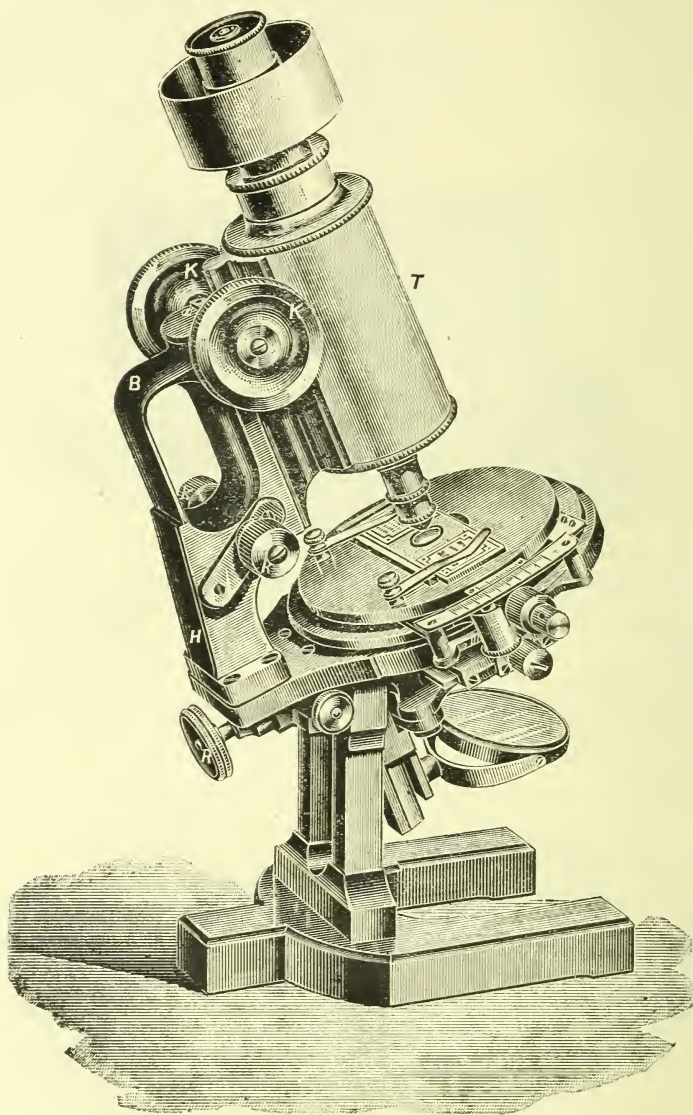
The oval-shaped piece B is arranged as a handle, which affords a strong brace between the fine adjustment path V (fig. 100) of the micrometer work and the special hollow-cast standard H (fig. 99). The fine adjustment differs widely from the general form, and is fitted by means

\* Zeitschr. f. wiss. Mikr., xiv. pp. 433-6 (2 figs.).

† Zeitschr. f. Instrumentenk., pp. 129-33 (3 figs.).

of a very strong dove-tail shaped slide F, so hollowed out in its upper part that it affords the requisite room for the spiral spring W. The

FIG. 98.



under part of F is pierced, and conceals the very long nut for the micro-meter-screw M. Contact ensues between the hard-tempered extremity

of the micrometer-screw and another also hard-tempered anvil-shaped steel piece which is screwed into the lid  $D_2$ , closing dust-tight the under

FIG. 93.

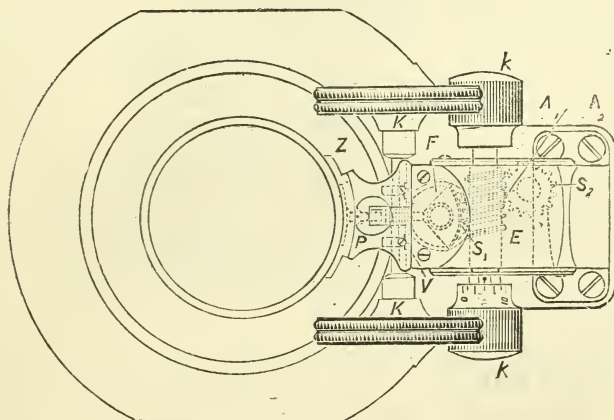
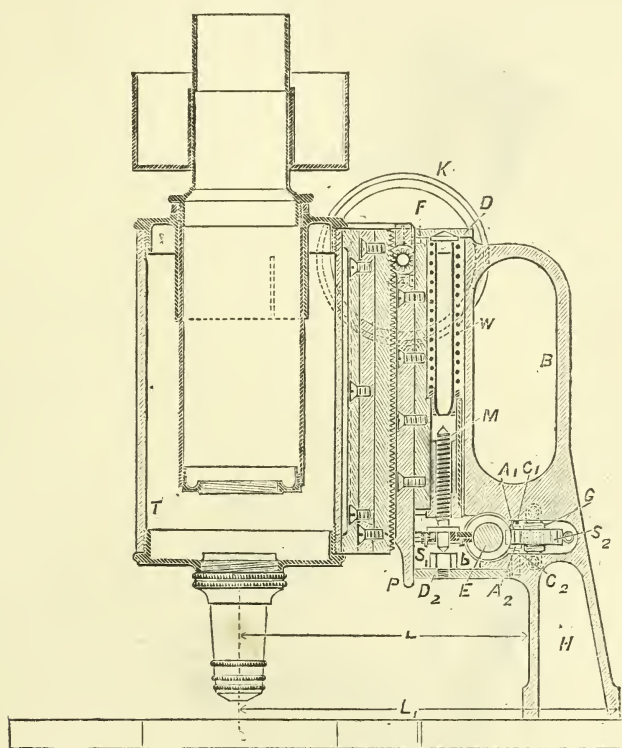
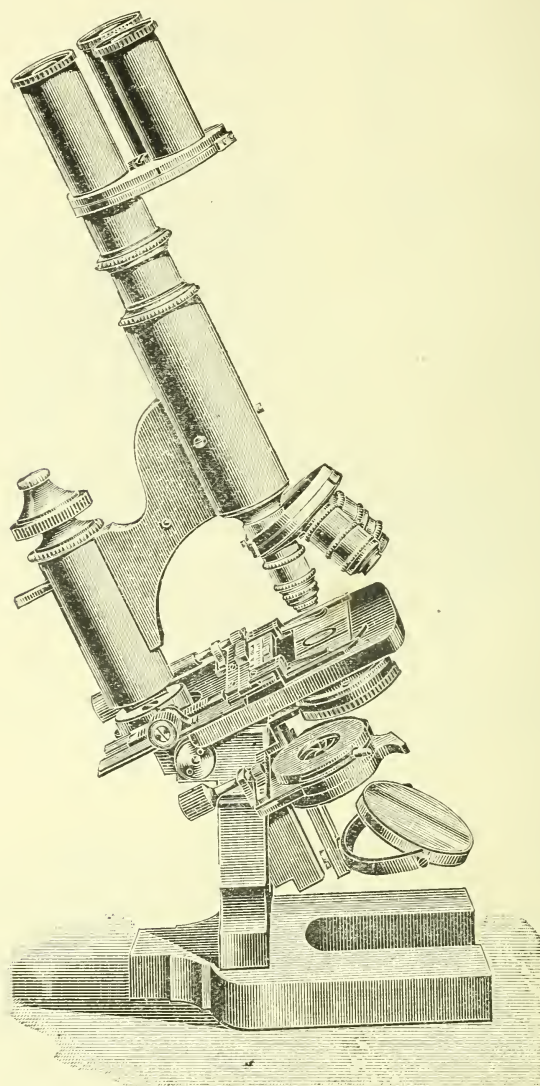


FIG. 100.



opening in the standard H. On the fine adjustment slide F rests, very solidly screwed, the gear P for the coarse movement. The aluminium tube T (fig. 99), in order to avoid undue wear and tear of the mechanism,

FIG. 101.



is connected in the usual way with the tooth-rack by means of a copper companion piece Z (fig. 100). The coarse adjustment follows the usual models. The micrometer-screw is thus seen to be packed away in the

hollow stand for protection's sake. It is completely sheltered from direct hand-touch. It is turned by means of an endless screw E (figs 99 and 100) which gears into the toothed wheel S, working on the flange of the screw, and carries the usual micrometer knob. By means of this arrangement two conditions are satisfied: the fine adjustment is slowed in the most desirable way without the necessity of requiring from the micrometer-screw too fine a motion, and the position of the endless screw, so firm and safe in the handle-like stand, renders even rough mechanical operations entirely without effect on the fine adjustment.

In order to avoid injury to the micrometer-screw when the slide F is at its extreme highest or lowest position, a special protection arrangement, limiting the play of the endless screw, is contrived for the fine adjustment. As is seen from fig. 100, the endless screw E engages another toothed wheel S<sub>2</sub> which is a nut working on a vertical screw. This nut S<sub>2</sub> comes into contact with the upper or lower surface of the chamber containing it before the fine adjustment slide F reaches the end of its travel, thus preventing strain on that part of the mechanism.

**Messter's Bacteria Microscope.\***—The makers of this instrument claim that its specially advantageous construction almost entirely removes troubles arising from loss of time (1) in changes of eye-pieces and objectives; (2) in tedious coarse adjustment for various powers; (3) in laborious picking out of very minute objects with strong magnifications. Investigations can therefore be made, even by a tyro, more quickly, accurately, and conveniently, with this instrument than with any other. It is specially recommended to physicians for diagnosis of urine and sputum. The figure (101) shows clearly the arrangement of the eye-pieces and objectives with their revolvers; and the fitting of these is so perfect that the image is always adjusted for every nine magnifications. [Thus apparently no coarse adjustment is required.—Ed.] An improved central micromillimetre-screw secures the fine adjustment; and a lever under the micrometer-screw effects a raising or lowering of the tube without change of adjustment, which is very convenient in the application of thick-ringed slides, or in the rotation of the objective revolver.

**Messter's Compressorium Microscope.†**—This is specially designed for the easy and certain discovery of trichinae and other objects.

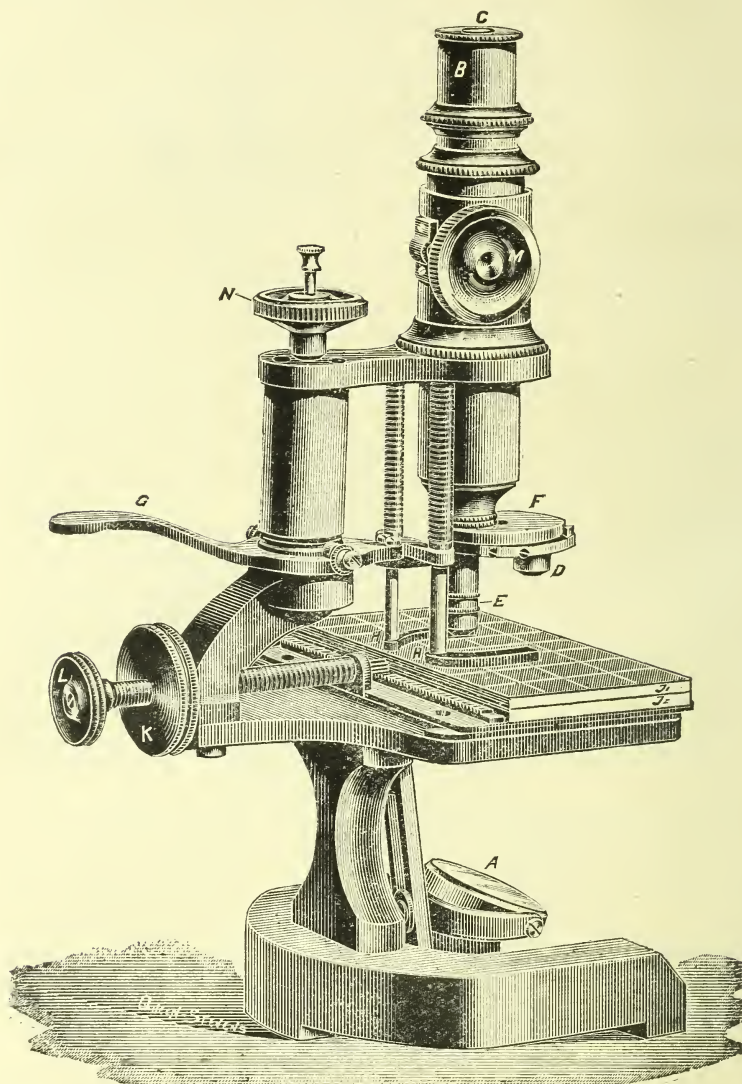
By pressing down the lever G (fig. 102), the pressure of the compressorium II on the glass plates J<sub>1</sub> and J<sub>2</sub> is released, and the table-screw L is drawn back to its fullest extent. This screw withdraws the runners on which rest the glass plates, and these last receive between them the slice of meat to be examined. When the object is ready the compressorium is again brought into play, and inspection is made by transmitted light with the weak objective. The outside corner of the field is at first under the Microscope; then, by rotation of the mother-screw K, the front line of numbered squares are brought one by one into view; L is now turned until the first of the next row is in sight, and, by reversing the mother-screw, the whole of the squares in the second line are inspected; thus in time the whole of the field is examined. When once found the revolver brings the high power into use. As the

\* Messter's Catalogue, p. 12.

† Cat. cit., p. 21.

objectives are accurately fitted on the revolver, adjustment is obtained by the micrometer-screw.

FIG. 102.



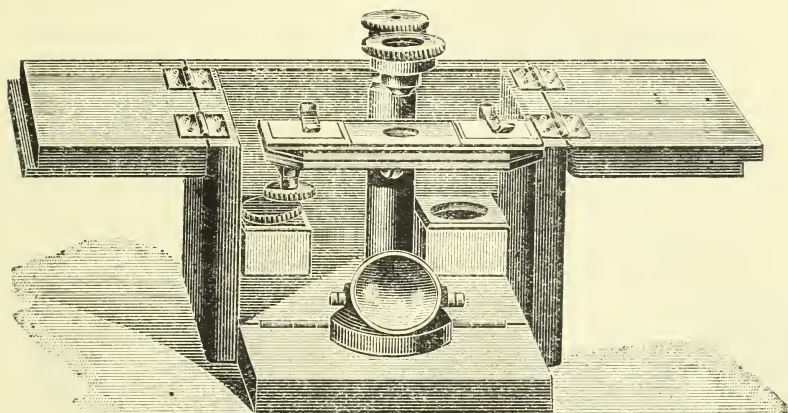
**Messter's Preparation Microscope.\***—In this class of simple Microscope, which seems intended for dissection or preliminary selection, the image of the object always presents itself in its natural relations. Hence

\* Messter's Catalogue, p. 37, No. 16.



difficulties arising from image-reversal are avoided. The lid of the box is constructed with flaps which, when folded back, form supports for the hands of the operator. The front lets down, and thus light is admitted to the mirror. Lenses of 20, 30, and 50 linear magnification are supplied.

FIG. 103.



The stand is fitted with a micrometer-screw, and the general arrangement will be readily understood from fig. 103.

A large size model of this instrument is also made with magnification up to 100 linear, with tooth-and-rack adjustment, and with plane and concave mirror.

### (3) Illuminating and other Apparatus.

**New Apparatus for Application of Electric Currents to Living Microscopic Objects.\***—Dr. Alfred Schaper describes the apparatus constructed to his designs by the firm of Zimmermann, Leipsic. His especial object was to secure that the object-holder should be free from polarising currents. He uses a piece of plate glass 12 by 7 cm., perforated at the centre with a circular hole 20 mm. in diameter. Along each of the narrow sides is cemented a strong nickelled metal plate 6 by 3 cm., furnished with binding screws as shown in fig. 104. This plate, which he calls the conductor, is placed on the object-stage so that the hole is immediately over the Abbe condenser.

The object-holder varies according to the kind of experiment and the nature and size of the object; but its general construction is such as to ensure communication of the current from the pole-plates. For this purpose both ends of the object-holder are mounted with a clamp-like metal piece which surrounds the edges of the holder and firmly unites its broad lower plane to its narrow upper one (figs. 105, 106, *m*).

\* Zeitschr. f. wiss. Mikr., xiv. (1898) pp. 436-41 (5 figs.).



When arranged as shown in fig. 107, on the conductor plate, the binding screws of the first lie exactly on a pole-plate, and are by means of the metallic contact themselves electrified. The dimensions are so chosen that the pole-plate can be moved as much as 2 cm. without losing contact. In this way the electrified and yet independent object-holder can be moved sufficiently without hindrance, so that Microscope examination can be effectively carried out.

On the upper side of the object-holder arrangements are made for the reception of the electrodes.

Fig. 105 shows a form adapted for the smallest organisms and strongest objectives; fig. 106, one for larger objects such as snails' eggs.

The former of these consists of an object-holder, as described above, with a shallow circular sinking, some 15 mm. in diameter, communicating on each side with a groove 2 mm. wide, in which lie the platinum thread poles of the electrodes. When this sinking and grooves are filled with

FIG. 101.

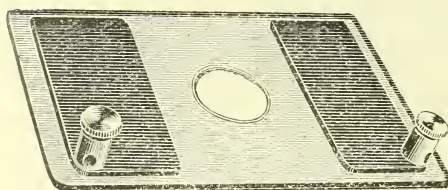


FIG. 105.

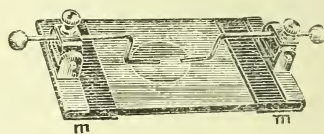


FIG. 106.

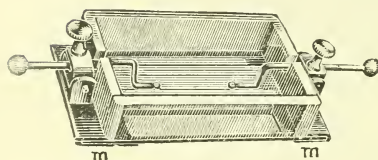
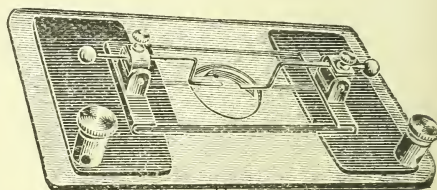


FIG. 107.



water, and a cover-glass applied, the platinum poles can be placed at any desired distance, and can even be pushed under the cover-glass. This form of pole gives an ellipsoidal electric field; platinum foil poles give a parallel electric field.

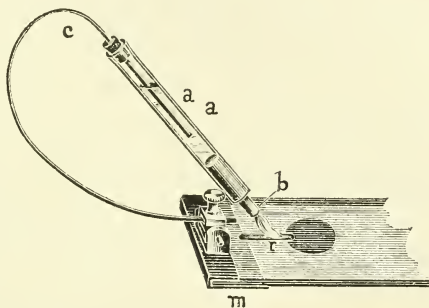
The second object-holder is shown in fig. 106, and is of the same size as the former, but bears in addition a glass rectangular trough 11 mm. high. Its sides have an insertion 3 mm. deep, through which the copper wire electrodes project into the interior of the trough. The poles consist of small platinum shanks, and the adjoining parts of the wires are coated with an insulating varnish. Two glass slips 4 mm. high and 6 mm. apart are cemented on to the floor of the trough parallel to the electrodes, so as to fix the object better in the current. The trough contains a relatively large amount of water, which is very desirable for the larger organisms. A glass cover can be adapted to the trough.

The above electrodes are naturally polarisable, but they answer well

for even a series of experiments if long exposure is not required or more than weak currents.

For long continuing investigations, especially when absolute exactness is desired, Flörsch's brush electrodes may be advantageously used. A narrow glass tube 4 or 5 cm. long is cemented at its lower end with plastic clay, through which passes a fine short brush (fig. 108, *a* and *b*) and the tube is three parts full of zinc sulphate solution. The upper end is closed with a cork, through which passes a strong zinc wire, so that its lower end dips 1 cm. into the solution. The part of the wire within the tube must be amalgamated. The other end of the wire is bent and connected with the binding screw. The whole arrangement must be so adjusted as to lie exactly central with the groove previously strewn with kaolin, and the kaolin is also slightly sprinkled into the central hollow of the object-holder. The brush point thus lies on the kaolin mass which now forms the unpolarisable poles. When used with the trough, the brush is fitted to the insertions in the side walls.

FIG. 108.



#### (4) Photomicrography.

**Photomicrography.\***—Mr. Edmund J. Spitta furnishes a series of papers on this subject, which he treats under three heads:—(1) Low-power work; (2) Medium-power work; (3) High-power work.

(1) *Low-power work.*—After pointing out that in this branch the Microscope is not used, he gives a useful series of practical directions. In discussing the choice of a lens he recommends one of short focal length. Where the object is large and the magnification required small, a diminutive portrait lens of about 3 in. focus, built on the Petzval principle and placed with its shorter conjugate focus towards the object, has given good results; but if the magnification exceeds  $1\frac{1}{2}$  to 2 diameters, it is difficult for the operator to reach the milled head. However, Messrs. Dallmeyer have specially constructed a small rectilinear lens of about  $1\frac{3}{4}$  in. focus, which, with magnifications over  $1\frac{1}{2}$  to 2 diameters, has given really admirable results; its only fault is that it is not constructed to work equally well with red, green, and violet rays. Mr. Spitta has found the finest type of lens to be the planar recently introduced by Zeiss, and constructed from calculations by Dr. Rudolph for the especial purpose of photomicrography. It works equally well on all colours of the spectrum, gives exquisite definition up to the margin of the plate, and is manufactured in several focal lengths. Its expensiveness is a matter for regret. An auxiliary front to the camera, attached where the lens usually fits, gives an increased extension of 6 or 8 in., and still

\* Pharm. Journal for 1898, pp. 326-9, 432-3, 474-5, 566-8, 587-9 (16 figs.).

allows focussing if the short-focus "planar" or Dallmeyer lens be used.

Among other details are some special directions for the microphotography of culture tubes.

The formula  $F = \frac{L}{M+1}$  is found to be sufficiently accurate for practical work ( $F$  = focal length of lens required,  $M$  = magnification, and  $L$  = camera-length). Thus, if it be required to produce a magnification of four diameters with a camera-length of 10 in., then  $F = \frac{10}{4+1} = 2$  in. In the same way, if any two of the three quantities  $F$ ,  $L$ ,  $M$  be known the third can be calculated.

(2 and 3) *Medium and High-power Work.*—In these sections the various difficulties to be encountered are fully described. The author's favourite Microscope is the Zeiss model I A, although apparently it is so badly balanced that it has to be clamped to the table to prevent it tumbling over.

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

**Microscopic Images and Vision.\***—Mr. Lewis Wright, from the point of view of the microscopist, continues the investigation of this subject on the lines commenced by Lord Rayleigh and Dr. Stoney.† His object is to show that the "spectrum" (i.e. the ordinary Abbe) theory is only true in a conditional and limited sense, while its acceptance in a universal sense is a present cause of positive mischief in microscopy. He begins by discussing some fundamental physical objections to Dr. Stoney's seven propositions, which propositions may be summarised thus:—(A) "All light emitted by an object may be resolved into undulations consisting of uniform plane waves." (B) "We may conceive these reversed in direction (since any dynamical system may be reversed); and when they thus arrive back at the position occupied by the original object, they will then produce an image the most perfect that the light emitted is capable of producing." Hence in general, "plane waves converging inwards" are capable of producing the most perfect attainable image producible from the rays grasped by the objective. Mr. Wright objects that uniform plane waves are not in trustworthy microscopy the actual dynamical system, and therefore cannot, as he afterwards shows they do not, produce the supposed most perfect attainable image by reversal. More specifically, it seems evident that we are debarred from considering the light from a microscopic object as consisting of uniform plane waves, *except on the condition of plane-wave illumination of the object.* (Here, indeed, we have the secret of Abbe's consistent enforcement of illumination by a small luminous cone or pencil, which gives approximately such illumination.) To get the normal wave-surface approximately plane, the beams of rays must be got approximately parallel, which is attained in physical optics by removing the source of light, itself relatively small, to a considerable distance from the grating or other apparatus. Now, in the case of a

\* Phil. Mag., June 1898, pp. 480-503 (1 fig.).

† Cf. this Journal, 1896, p. 681, and 1897, p. 71.



microscopic objective of focus  $1/50$  in., the light would travel a path of perhaps  $1/200$  in. from the object to the lens, and such light cannot be regarded as consisting of uniform plane waves, except in the case of plane-wave illumination of the object, as in the Abbe theory.

In discussing the supposed dynamical system, Mr. Wright admits that a reversal of the *whole* actual system would produce such an image as described; but it does not seem to follow that mere "coalescence and interference of uniform plane waves" involves such a result. In any case, what the reversal of the supposed dynamical system must really reproduce as an image at the plane of its origin, must be the postulated operative cause of the system. That cause, by the hypothesis, is not an actual object which alone emits luminous waves, but *the object surrounded by an indefinite number of identical replicas, emitting identically similar plane waves*. This does not represent any object in reality; and that fact seems to dispose of such a presentment as a full and complete representation of microscopic vision.

In considering how far the Abbe theory, which possesses more or less undoubted truth, is an adequate representation of microscopic vision, the author states his own opinion thus:—"The trustworthiness of a microscopic image is in proportion as the object approximates to a self-luminous condition, and diminishes in proportion as it is or has to be (for it may have to be) examined by plane-wave illumination."

If the object be self-luminous there will be no spectra, and the rays emitted will be quite heterogeneous; yet an image must be possible. Really self-luminous objects can hardly be used with objectives above an inch, but approximations to self-luminosity can be obtained in various ways with high powers. Thus Lord Rayleigh has shown that the wide cone from a condenser introduces a large amount of heterogeneity into the rays from the objects, and practically produces self-luminosity. Dr. Stoney rather seems to regard the function of the condenser as that of providing illumination by plane waves. Thus, according to Dr. Stoney, the ideal is to get absolutely aplanatic systems of plane waves transmitted through the objects, and all conditions short of this impair the image; but according to the author, irregularities of phase add to the *trustworthiness* of the image, though they may impair it in some other features. A very important practical question is therefore at stake.

The following experiment is one of those by which the author proves his view. He takes as his stage object a grating of 3000 or 6000 lines to an inch, illuminated by a narrow cone from the condenser, focussing the flat of a rather distant lamp-flame. Immediately in front of this flame he places a coarse grating of 50 to 100 lines per inch, either photographed or of wire. The several points of these luminous lines emit light-waves, chiefly in the self-luminous manner, indiscriminate in phases and transversals as the points of the flame itself. Arranging the stage grating so as to cover only half the objective field, a condenser can be selected of such a focal length, and other matters so adjusted, that the focal image of the coarse grating formed by the condenser corresponds, both in intervals and focal plane, with the object-grating on the stage, the same illuminating cone being used. It now remains to move the coarse grating, and place the stage grating centrally; then, on removing the eye-piece, and looking down the tube, the dioptric beam or its flanking spectra,



as so often described, will be seen. They interfere, and form the image seen by the eye-piece in the Fresnel and Abbe manner. Removing the stage-grating, and replacing the coarse one over the flame, its focal image is now the object. Owing to the heterogeneity of the rays, this aerial image emits no spectra—there neither are nor can be any such. But it is perfectly resolved. Here we have a resolution of 3000 or 6000 lines per inch that has no place at all in the “spectrum” theory; which, therefore, can be no complete theory of microscopic vision, though it has an important place in it.

It is highly desirable to find out what proportion and value must be assigned to the Abbe image in ordinary research. There are really two factors in the standard image, which is the outcome partly of the features upon the object, and partly of the state of the light by which the object is illuminated; and Dr. Stoney himself says, “It (the image) may be improved by increasing the degree in which the first of these factors, and by decreasing the degree in which the second, contributes to produce, to modify, or to efface detail in the image.” With this statement the author cordially agrees, but differs from Dr. Stoney in the estimate of the true relative proportions of the factors. Thus the results of diatom photomicrography on Abbe methods are woefully inferior.

Wherever we have a known periodic structure in transparent objects, plane wave illumination and the consequent interference lines formed by the beams diffracted by that structure have an extraordinary effect in *intensifying* into black and white a more or less accurate representation of the periodic detail. (Two experiments illustrating this are described.) Thus the Abbe method has a most important function in enabling us to see *contrast* in the details of a large class of objects—especially hyaline or transparent objects—which do not present contrast or opacity sufficient to be seen in any other way. The error has been in giving to it the sole or all-important place, not recognising that there is quite another kind of image also available, depending upon Airy’s theory; and that the latter—while in the case of transparent details often giving images insufficient, or at least far inferior, in black-and-white contrast—is free from the *contour* errors of the Abbe image, and must be used to correct it so far as is possible in the individual cases. The errors of the “spectrum” image are well known. Its very contrast or “resolution” is a departure from truth, to which the more indistinct self-luminous image is in reality a much nearer approach. It tends to make details, which should be only geometrically symmetrical to a limited extent, perfectly so. All that can be really learnt from it is that there is probably some periodic difference of structure in the object similar in dimensional intervals to “lines” shown; less probably so in regard to “spots,” since these are often produced by false diffraction fringes from any long line which may cross the true ones. That the lines are lines, or that the “pattern” is so geometrical as appears, is in the highest degree improbable. That the “spectrum” theory and method so long retained exclusive predominance is because attention has been so concentrated upon either gratings or diatoms of known periodicity in structure, but which only represent to a very small extent indeed any serious kind of investigation.

It thus appears that in Microscopy we have to deal with two charac-

teristics of an image, which often are only to a limited extent compatible; that we have at command two methods of illumination, which respectively promote more especially each of such characteristics; and that in most cases our problem is so to combine and balance these two methods as to produce the best result. *Fidelity of contour* will be secured in proportion as we are able to obtain our image by heterogeneous illumination, approximating the object to a self-luminous condition. But this method may prove utterly unable to give us *contrast*, which we may therefore have to gain at the expense perhaps of infidelity in contour. Thus an opaque object, even of much minuteness, may be best shown by ground-glass illumination, or a very wide cone; while a diatom, unless in a very dense medium, or dry in air, may require narrow pencils of approximately plane waves. Moreover, neither kind of pencil can be obtained absolutely pure. The narrowest pencil will only approximately consist of plane waves, and the widest cone will not be free from them. We thus understand why, in really critical work, a large cone from a good condenser usually gives us the best result; but why it may be impossible, even with a perfect objective, to use a cone of light which will fill its aperture completely. It may be necessary to intensify the image, while using as much heterogeneous light as we can. But this necessity depends on the nature of the object, and does not exist in all cases.

There is a very obvious and simple, yet decisive test as to the correctness of this view. According to the Abbe theory, the amount of cone light which can be used will depend upon the *minuteness* of the structure alone. According to the author's view, the *density or contrast* of the structure is the chief factor in this question. All experience proves that the latter is the case.

In conclusion, the author discusses the effect of these theories on the work of the Microscope optician, and on the prospects of further microscopical research. Devotion to the Abbe theory alone has even led Continental microscopists to challenge the importance of spherical aberration in objectives; but Mr. Wright shows that the perfect correction of spherical aberration of a lens determines how far we may go in the use of the heterogeneous cone with that lens. The well-known fact that individual similar lenses frequently possess marked superiority, shows a more perfectly corrected spherical aberration; and this suggests the direction of further improvements. It is not so much further minuteness, but greater powers of resolution within the limits of our present lenses, that are desiderated.

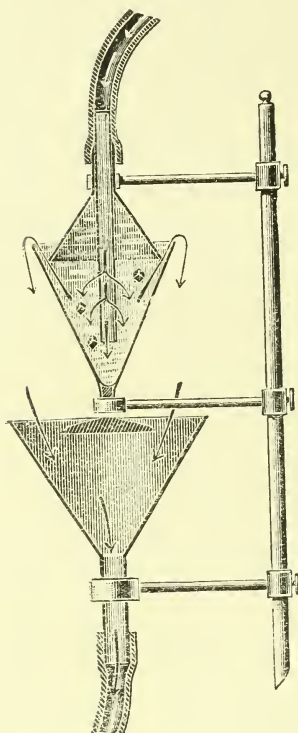
#### (6) Miscellaneous.

**Simple Apparatus for Washing Microscopical Objects.\***—Dr. G. Cruz describes an apparatus (fig. 109), which he uses for washing tissues, &c., intended for microscopical examination. The apparatus consists of a glass funnel fixed to the upright of a laboratory stand. Above this is another funnel in the reverse position. The upper funnel, also fixed to the upright, is of less diameter than the lower one. Through its stem passes, to near the bottom of the hopper of the lower funnel,

\* Zeitschr. f. wiss. Mikr., xv. (1898) pp. 20-30 (1 fig.).

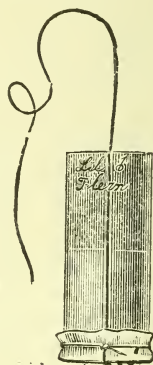
a glass tube, the end of which is perforated in several places. The stem of the upper funnel is connected with the water supply. The overflow is caught in a still larger funnel, and passes away to a sink through the stem.

FIG. 109.



**Apparatus for Fixing and Hardening Small Objects.\***—Mr. W. C. Stevens recommends the following method for carrying material through the processes of fixing and hardening. For very small objects such as root-tips, sporanges, and young flower-buds, it is specially useful. Small glass buckets are made by cutting up glass tubing 1 cm. in diameter into lengths of 3 cm. By means of heat one end of each piece

FIG. 110.



is turned out so as to form a rim or flange. Over this end a piece of muslin is tied. The little bucket is then provided with a suspender by means of a piece of thread fixed to the middle of the bottom (see fig. 110). If, when the bucket containing the specimen is suspended in the fixative fluid, the bucket does not sink, a weight should be attached, or the air may be removed from the specimen by means of the air-pump.

**Thermo-regulated Water-baths for the Bacteriological Laboratory.†**—Mr. V. A. Moore describes two water-baths which have been found to work very satisfactorily.

The larger bath (fig. 111), to which a thermostat is connected, is a rectangular copper tank  $40 \times 50 \times 25$  centimetres. It is divided into two compartments, each of which has a separate cover and perforated false bottom. The partition consists simply of a top cross-piece which is about 4 cm. wide. Near its centre is a round opening 2 cm. in

\* Kansas Univ. Quarterly, vii. (1898) pp. 107-10 (3 figs.).

† Journ. Applied Microscopy, i. (1898) pp. 108-9 (2 figs.).



diameter for a thermometer, which is protected by a perforated copper tube extending to and soldered to the bottom of the tank.

Near the end of the cross-piece or close to the side of the tank there is a second and similar opening and shield for a thermostat. There is a faucet for drawing off the water. The tank stands upon an iron quadruped. Each compartment is heated by a separate burner connected by a T or Y tube to the gas tube leading from the regulator.

The smaller bath (fig. 112) is cylindrical in form, 25 cm. high and of about the same diameter. On one side there is a semicircular projection forming a chamber for the thermostat. This is separated from

FIG. 111.

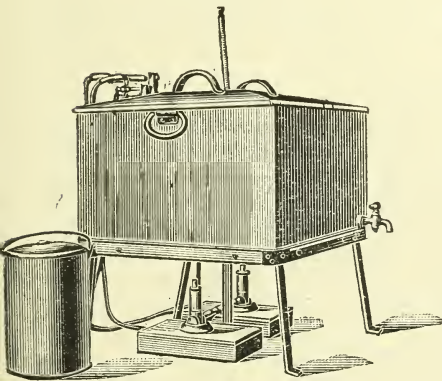
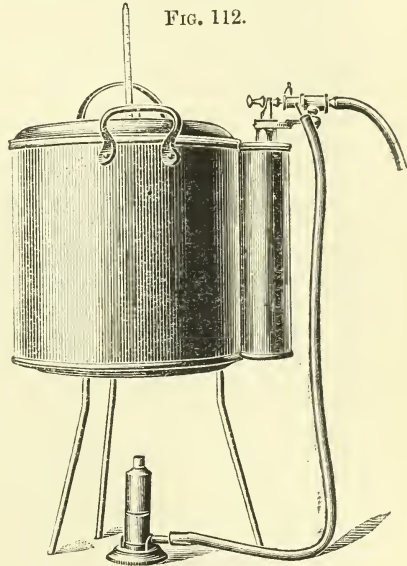


FIG. 112.



the main tank by means of several narrow strips of copper soldered at each side. The tank has a perforated false bottom. The cover has an opening for a thermometer.

The Friedberg burner was found to work very satisfactorily with both baths. The Roux thermostat seemed to be the best regulator; it is constructed of metal, is readily adjusted, and quite as sensitive as spirit or mercury thermo-regulators.

The larger bath can be used for macerating meat at a high temperature ( $60^{\circ}$ – $65^{\circ}$ ), for making culture media, for sterilising, for determining the thermal death-points for different bacteria, and for other purposes.

### B. Technique.\*

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

**New Medium for Bacteriological Diagnosis.**†—Sigg, Pacinotti and Munciechi have devised a coloured nutritive medium which may aid in

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

† Zeitschr. f. angew. Mikr., iv. (1898) pp. 106–7.



the diagnosis of different kinds of bacteria. The medium is made by mixing 140 grm. of egg-albumen with 20 grm. of powdered raw coffee. After standing for 2 or 3 days, the mixture assumes an emerald-green hue, which afterwards becomes darker. After filtering through gauze into test-tubes, the medium is sterilised for 2 hours a day for several consecutive days and allowed to set. On this medium the bacteria do not become tinted, but the colour around the colonies becomes altered. Colonies of *Meningococcus* and *Pneumococcus* are surrounded by an orange-yellow zone. Those of *St. py. aureus* produce a pale yellow hue, with slight liquefaction of the medium; while *Proteus vulgaris* strongly liquefies the medium with the formation of a red colour. The staining of the albumen cannot be referred to the chlorophyll contained in the coffee-berries, for this is easily soluble in alcohol, ether, and benzin, and insoluble in water. The green coloration is the more inexplicable as the reaction is not acid. Mineral acids turn the green albumen reddish, but do not decolorise.  $\text{CO}_2$  has no action, and O turns it darker. If the cultures be killed by heating to  $100^\circ$ , or moistened with 1 per thousand sublimate solution, the original colour returns.

**Use of Acetylene in the Cultivation of Anaerobic Bacteria.\*—**Dr. J. Ferrán recommends the use of acetylene for cultures of anaerobic bacteria. The air in the culture vessels is driven out by means of the gas, which is easily prepared. The apparatus for making acetylene gas is composed of a 2-litre vessel half filled with water, and closed with a caoutchouc plug having two perforations. Through one of the holes passes a glass rod, having at the end a hook on which is suspended a little basket containing calcium carbide. Through the other hole passes a glass tube, connected with a rubber tube, and this latter with the culture vessel. The culture vessel is also closed with a doubly perforated stopper. Through one of the holes passes a tube for the exit of air, while the other is connected with the rubber tube from the acetylene apparatus. When the connection is made the calcium carbide is immersed in the water, and the gas at once develops. As soon as the air is completely driven out, the two tubes of the culture vessel are closed, and the calcium carbide drawn up out of the water.

**Culture of Diatoms.†—**Herr J. Burger communicates the following simple method for cultivating diatoms (*Gomphonema*). The material was placed in a glass vessel containing tap-water, some straw, bran, and moss. This vessel was covered with tissue paper, and hung up before the window in a moderately warm room. The culture was started in February, and in April the straw was found to be covered with diatoms.

## (2) Preparing Objects.

**Detection of Protoplasmic Threads in the Cell-wall.‡—**Mr. W. Gardiner gives further details of his two methods for demonstrating the connecting threads which traverse the walls of plant cells.

(1) The Kolossow safranin method, in which the tissue is first killed and swelled with picric acid, picrosulphuric acid, picro-acetic

\* Centralbl. Bakt. u. Par., 1<sup>te</sup> Abt., xxiv. (1898) p. 29. ;

† Zeitschr. f. angew. Mikr., iv. (1898) p. 61.

‡ Proc. Cambridge Phil. Soc., ix. (1898) pp. 504-12. Cf. this Journal, ante, p. 210.

acid, or in certain cases with sulphuric acid. It is then fixed with Kolossow's mixture of uranium nitrate and osmic acid. The material may be preserved in thymol water (0.5 gm. to 1 litre). The sections are stained with a saturated aqueous solution of safranin or with anilin-oil-safranin, and, after having been washed with water are treated with a 2 per cent. solution of orange G, which dissolves the dye from the cell-wall and leaves the protoplasm and connecting threads stained. The threads may now be further stained by substitution, gentian-violet and Gram's method giving the best results. After either the dye should be removed from the cell-wall by means of a 5 per cent. solution of acid fuchsin. After immersion in dilute glycerin, the sections are mounted in glycerin-jelly.

(2) Iodine-acid violet method. The tissues are killed and fixed with iodo-potassic iodide solution, with an average strength of 0.5 per cent. iodine and 0.75 per cent. potassic iodide. The sections are caused to swell by immersion in sulphuric acid varying in strength according to the tissue from 1 to 30 per cent. The sections to be stained are first mordanted with a solution of iodine in 5 per cent. sulphuric acid. The staining solution is made by mixing equal parts of two stock solutions, the first being a 10 per cent. solution of  $H_2SO_4$ , and the second a 0.5-1 per cent. solution of pyoktanin or gentian-violet in water.

To this staining solution the sections are directly transferred from iodo-potassic iodine and sulphuric acid mixture. The staining solution is allowed to act for 10 minutes, and then the sections are removed, and washed in water. The staining may be intensified by repeating the process.

The sections should be mounted in glycerin containing a small percentage of zinc chloride and a trace of iodine.

The mounting medium is made by mixing 30 ccm. glycerin, 60 ccm. water, and 10 ccm. of a 20 per cent. solution of zinc chloride, adding a flake or two of iodine, and heating over a warm bath.

**Preparing Agar Media.\***—Dr. M. P. Ravenel gives the two following simple and rapid methods for preparing agar media. (i.) To make 1 litre of agar, take (A) dried pepton (1 per cent.), 10 gm.; salt (0.5 per cent.), 5 gm.; Liebig's ext. (0.5 per cent.), 5 gm.; water, 500 ccm. Boil for 3 minutes, and neutralise. (B) Agar (1.2 per cent.), 12 gm.; water, 500 ccm. Chop the agar and put into autoclave. Run the autoclave up to 2 atmospheres, giving  $121.4^\circ$  heat. As soon as this pressure is reached, turn out the flame and allow the autoclave to cool down to below  $100^\circ$  before opening. The two solutions A and B are then mixed, cooled to  $60^\circ$ , the whites of two eggs beaten in 50 ccm. of water added, well stirred in, and the whole then boiled, and filtered through paper. Time, an hour and a quarter to an hour and a half.

(ii.) To make permanently clear agar, fresh meat should be used as follows. To make 1 litre take (A) chopped meat, 500 gm.; water, 500 ccm. Mix, and place in a cool place over-night, then strain through towel. (B) Agar (1.2 per cent.), 12 gm.; water, 500 ccm. Put in autoclave, run up to 2 atmospheres, put out flame, and cool down to below  $100^\circ$  before opening. When the agar solution has cooled down to about  $75^\circ$ , mix

\* Journ. Applied Microscopy, i. (1898) p. 106.

*A* and *B* together, add 10 grm. (1 per cent.) dried pepton and 5 grm. (0·5 per cent.) salt, boil for 3 minutes, neutralise, and filter. The product is an absolutely clear jelly, which never forms any precipitate. The filtration takes 10–12 minutes through an ordinary filter-paper. Solution *B* should not be added to *A* until cool enough to avoid coagulation.

**Treatment of Celloidin Sections Stained with Orcein.\***—Herr H. Jordan takes the celloidin-sections (80–96 per cent.), and by means of tissue paper places them on the slide already smeared with albumen or albumen-glycerin. The paper is firmly smoothed down, and on the top of it is placed another slide. The slides, firmly held in the hand, are then heated to coagulate the albumen. When this is effected, the whole is immersed in 96 per cent. alcohol, and the upper slide and paper removed. The preparation is then transferred to the solvent. The solvents recommended are a mixture of equal parts of absolute alcohol and ether or acetic ether. The latter may be used before or after staining, as it does not damage the orcein.

**Modification of the Weigert-Pal Method for Paraffin Sections.†**—Dr. E. E. Laslett recommends the following procedure as efficient and trustworthy. The material, spinal cord, medulla, &c., is hardened for about a fortnight in Müller's fluid, and then cut into slices not more than 2 mm. thick. These are placed in Marchi's fluid for a week, washed, and imbedded in paraffin. The sections are fixed to the slide by the water method, and then, after removal of the paraffin, placed in the acetic acid-hæmatoxylin over-night, preferably in a warm oven, as thereby the staining process is materially hastened. After washing they may be passed into a saturated solution of sodium or lithium carbonate, by which the colour is changed to a bluish black. They are then differentiated by the Pal method, care being taken not to over-decolorise. The advantages of this method are, firstly, that it is a combination of the Marchi and Weigert methods, and secondly, that the sections are much thinner than by the celloidin method.

**Method of Demonstrating the Structure of Yeast-Cells.‡**—In their investigations on the yeast-cells, Prof. F. A. Janssens and M. A. Leblanc used Moeller's iodine fluid for fixing, and alcohol at 95° for hardening. The iodine solution consists of distilled water 100 ccm., iodide of potassium 1 grm., iodine to saturation. A few drops of the iodine solution are placed on a slide, and a loopful of yeast culture carefully mixed with it. Some of the mixture is then carefully spread on cover-glasses. When the fluid has evaporated, the cover-glasses are transferred to fresh Moeller's solution for 24 hours. Then, after having been washed in water, they are passed successively through one-third alcohol, alcohol at 80°, and finally at 95° for at least two days. It is necessary to remove all traces of iodine. Several methods were used for staining the preparations, among the best being the following:—fuchsin 4 grm., phenol 10 ccm., alcohol 40 ccm., water 200 ccm. The preparations are decolorised with weak  $\text{H}_2\text{SO}_4$ , and contrast-stained with Loeffler's methylen-blue. The method which gave the best results con-

\* Zeitschr. f. wiss. Mikr., xv. (1898) pp. 53–5. † Lancet, 1898, ii. pp. 321–2.

‡ La Cellule, xiv. (1898) pp. 203–9. Cf. *ante*, p. 570.



sisted in mordanting the preparations for four hours in ferric alum 2.5 grm., water 100 cem., and then staining for 18 hours in hæmatoxylin 0.5 grm., water 100 cem. The preparations should be decolorised to the extent that the nucleus is quite black and the cytoplasm unstained.

Black hæmatoxylin was also used for staining. Preparations stained with this fluid may be advantageously contrast-stained with crocein, or by decolorising with orange or carmin-blue. In fact the cytoplasm may be completely decolorised with 0.1 grm. of carmin-blue dissolved in 500 cem. of alcohol at 80°. The preparations may be mounted in glycerin (50 per cent.) This fluid, while very suitable for examining the objects, is very detrimental to the stains, as the yeasts are more or less rapidly decolorised. The authors, after having examined and drawn the preparations, mount them in dammar-colophon (resin), after having contrast-stained them with Congo or Bordeaux red, crocein, or carmin-blue.

The cover-glasses must not be warmed, or the yeasts will shrink to about half their former bulk. In connection with these preparations, the value of amylie alcohol is noted. After the preparations have been passed through a series of alcohols up to 95°, a drop of amylie alcohol is placed on the cover-glass. This fluid, being denser than ordinary alcohol, forms a layer immediately beneath the latter and above the specimens, and, though insoluble in water, is extremely effective in dehydrating the yeasts, and also in preventing their absorption of moisture.

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

**Imbedding and Staining Lichens.\***—Mr. G. H. French recommends that lichens should first be immersed in 95 per cent. alcohol for 24 hours, and then placed in thin celloidin and thick celloidin for 24 hours each. The specimens are imbedded in thick celloidin, which is hardened in 70 per cent. alcohol for 24 hours, and then cut. Staining with borax-carmin gives the fungus part of the lichen a pale pink hue, while the algal cells have a greenish-red shade. In this way the host-cells are readily distinguished from the fungus.

### (4) Staining and Injecting.

**Methylen-Blue for Investigating Respiration in Plants.†**—Prof. J. B. Farmer points out the usefulness of methylen-blue for demonstrating the reducing power of living protoplasm. If germinating seedlings of barley or peas be placed in a test-tube filled with a 0.0005 per cent. solution of methylen-blue which has been boiled to expel the air, the liquid around them will in the course of a few hours be decolorised. If some of the decolorised fluid be withdrawn with a pipette and shaken up with air, the blue tint speedily returns. Still more striking results were obtained by putting *Chara* cells in the dark in the methylen-blue solution.

With many plants the reaction is tardy, and with all the result is attained more quickly if the plants have previously been starved of oxygen. It seems probable that the oxygen obtained by reducing the

\* Journ. Applied Microscopy, i. (1898) p. 135.

† Nature, lviii. (1898) pp. 185-6.



anilin dye is not directly utilised by the protoplasm, but by some dissociation product formed during the metabolic activity of the protoplasm.

**Testing Butter and Milk for Tubercle Bacilli.\***—Dr. Petri found, in 102 samples of butter, tubercle bacilli present in 32·3 per cent. The tuberculoid bacillus was also frequently present, both alone and in conjunction with the true tubercle bacillus. The test applied was to inject 5 ccm. of the butter into guinea-pigs. The animals which succumbed were carefully examined, and those which survived were killed and also inspected. If rodlets giving the characteristic staining reaction were found, further inoculations were made with the suspected material. While the true tubercle bacillus invariably gave rise to infection, the tuberculoid rodlet failed to excite specific inflammatory changes, and the inoculation wound healed up perfectly.

In 64 samples of milk, 14 per cent. contained tubercle bacilli and 6·3 per cent. the tuberculoid rodlet. The milk used was centrifuged, and 3 ccm. of the cream, the buttermilk, and the sediment were injected into the peritoneal sac of four guinea-pigs.

**Black Hæmatoxylin.†**—Prof. F. A. Janssens recommends the following preparation of hæmatoxylin for biological work. It is prepared very much in the same way as Delafield's hæmatoxylin, the chief difference being that iron-alum is substituted for ammonia-alum. To a hot saturated solution of ammonia-iron-alum  $(\text{NH}_4)_2\text{Fe}_2(\text{SO}_4)_4$ , are added 4 grm. of hæmatoxylin dissolved in absolute alcohol. The soluble black dye which is thereby formed is allowed to stand for a while, and then 100 ccm. of glycerin and 100 ccm. of methyl-alcohol,  $\text{CH}_3\text{O}$ , are added.

**Prodigiosin as Staining Reagent for Botanical Specimens.‡**—Prodigiosin, the pigment produced by *Bacillus prodigiosus*, is recommended by Herr O. Rosenberg as a useful reagent for staining cuticula, corky membranes, and fatty substances. To these it imparts a deep red colour, and though woody tissue and cell-contents are also faintly stained, they lose the stain when washed in alcohol. The pigment is obtained by cultivating the microbe on potato at 25° for 3 or 4 days. The bacterial mass is then scraped off, placed in a glass vessel, and dissolved in 95 per cent. alcohol. The alcoholic extract when filtered is a clear brick-red fluid. For staining sections the strength recommended is 5 ccm. of the bacterial substance to 25–30 ccm. alcohol. Exposure to light damages the colour; but if preserved in black bottles protected from light, the fluid will keep for a good time. The sections from fresh or preserved tissue are immersed in the fluid for 5 to 10 minutes, and on removal it is well to pass them through alcohol. The parts stained are cuticula and corky tissue, though the oil-drops in the hyphæ of fungi take up the pigment freely. It is easy to double stain with prodigiosin, especially with malachite-green or chloranilin. In the former case the woody tissue is green; in the latter yellow.

\* Arb. aus d. kaiserl. Gesundheitsamt, xiv. (1898) p. 1. See Zeitschr. f. angew. Mikr., iv. (1898) pp. 64–5.

† La Cellule, xiv. (1898) p. 207.

‡ Zeitschr. f. wiss. Mikr., xv. (1898) pp. 56–60.

**Staining the Mucus-cells of Malvaceæ.\***—Herr A. Nestler used Böhmer's hæmatoxylin for demonstrating mucus, the mucus-cells being stained blue. Alcoholic solution and Loeffler's solution of methylen-blue also stained the mucus-cells blue. With Meyer's reagent a similar result was obtained. The sections from material taken from alcohol were placed in 25 per cent. solution of copper sulphate, washed with distilled water, and treated with 50 per cent. caustic potash. The mucus in Malvaceæ swells up in copper-ammonium oxide and becomes pale blue. With tincture of iodine and iodopotassic iodide there is no reaction; with alcoholic solution of safranin it turns orange, and with tincture of alkanna steel-blue.

**Diagnostic Stain for the Diphtheria Bacillus.†**—Dr. R. T. Hewlett communicates Neisser's differential method for staining the diphtheria bacillus. (i.) One grm. of methylen-blue is dissolved in 20 ccm. of 96 per cent. alcohol and mixed with 950 ccm. of distilled water and 50 ccm. of glacial acetic acid. (ii.) Two grm. of benzoin are dissolved in 1000 ccm. of boiling distilled water, and the solution filtered. Cover-glass films from fresh serum cultures are stained in No. i. for 1–3 seconds, rinsed in water, counter-stained in No. ii. for 3–5 seconds, washed, dried, and mounted in balsam. Thus treated, the diphtheria bacillus shows as a brown-stained rodlet containing dark blue granules, usually polar, but occasionally with one at the middle of the rod.

The method is not so successful with swabbings directly from the throat, but with fresh membrane it affords a rapid means for positive diagnosis.

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

**Mounted Specimens—a New Departure.**—Mr. C. Baker, of 244 High Holborn, proposes during the ensuing season, should the project receive sufficient support, starting a department for lending specimens, much on the same lines as a lending library. Should the undertaking prove a success, Mr. Baker has arranged for the subscription to include postage both ways, as detailed below. The annual subscription will be 17. 1s., payable in advance. For this sum each subscriber will have the right of borrowing some 250 specimens in 12 deliveries; that is to say, 24 slides posted monthly for the year, or the same number of slides posted each fortnight for the winter months only. In order to save subscribers as much trouble as possible, it is proposed to send the specimens in special boxes with double wrappings, the inner one of which will be stamped and addressed for return, so that the subscriber has only to re-wrap the parcel and post it. Should sufficient subscribers send in their names, steps will at once be taken to compile a catalogue especially for this department, so that subscribers may choose as far as possible the specimens they wish to see. This, however, would not be possible until the undertaking were an assured success.

Those desiring to subscribe should communicate at once with Mr. C. Baker, Optical Warehouse, 244 High Holborn, London, W.C.

\* Oesterr. Bot. Zeitschr., xlviii. (1898) p. 94. Cf. this Journal, *ante*, p. 440.

† Brit. Med. Journ., 1898, ii. p. 599; and also Zeitschr. f. Hyg., xxiv. (1897) p. 443.

## (6) Miscellaneous.

Some Methods of Determining the Positive or Negative Character of Mineral Plates in Converging Polarised Light with the Petrographical Microscope.\*—Dr. M. E. Wadsworth gives the following instructions in the use of the petrographical Microscope as a polariscope.

"Since by varying the powers, the petrographical Microscope can be used with mineral plates of any standard thickness, the directions here given can be used with the ordinary polariscope plates, as well as those thinner ones prepared expressly for use with the Microscope.

I. *Uniaxial Minerals*.—When the mineral plate shows the common uniaxial cross in converging light, its positive or negative character can be ascertained by means of the gypsum plate or quartz wedge, as well as by the ordinary mica plate.

(1) Use of the Gypsum Plate.—Examine the mineral plate, which, in converging polarised light between crossed nicols, shows a dark cross or part of a cross with or without coloured rings or arcs. Insert the gypsum plate in the slot in the body of the Microscope, above the objective. The cross is then resolved into coloured hyperbolas. The central portion is red, terminated on the ends with yellow, and bordered on the side by blue. If the blue that borders the red lies on a line parallel to the axis of least elasticity, the mineral is positive; but if it lies on opposite sides of this line, the mineral is negative. The gypsum plate is often more satisfactory in its use than the mica plate for these determinations.

(2) Use of the Quartz Wedge.—Insert the quartz wedge thin end forward. When the wedge is gradually pushed in, the cross resolves itself into coloured arcs that cross the field of view from two opposite sides of the field, and pass out of sight on the other two sides. These arcs follow each other in succession as the wedge is pushed in. If these coloured arcs advance towards the centre of a line parallel to the axis of least elasticity, the mineral is positive; but if they march toward the centre from opposite sides of that line, the mineral is negative.

The use of the quartz wedge is less liable to error than either of the preceding, and besides it can be used in many cases where the others give no results.

(a) If the uniaxial plate is cut so that it shows arcs of rings, its positive or negative character can be determined by placing the arcs so that a line perpendicular to them shall make an angle of  $45^\circ$  with the cross-hairs. By use of the quartz wedge, coloured arcs or rings can often be brought into the field, when otherwise none are seen. Push in the quartz wedge with its axis of least elasticity tangent to the arcs. If the rings then move outwards with their convex side forward; and, in time, a black or partially black arc appears, the mineral is positive; but if the arcs move with their concave sides forwards, the mineral is negative.

As a check against any error, turn the wedge over and push it in, so that its axis of least elasticity will be perpendicular to the arcs. If then the arcs move with the concave side forward, the mineral is positive;

\* Journ. of Applied Microscopy, i. (1898) pp. 20-1.



but if they move with the convex side forwards, and a black or partially black ring or rings show, the mineral is negative.

(b) A uniaxial plate cut parallel to the vertical axis can have its positive or negative character shown in converging polarised light as follows. Place the plate at an angle of  $45^\circ$  with the cross-hairs so as to show the coloured arcs or imperfect hyperbolas. Push in the quartz first with the axis of least elasticity perpendicular to the vertical or optic axis of the plate. If on pushing along the quartz wedge a dark hyperbola is seen to pass over the field, the mineral is positive. Again, push in the quartz wedge with its axis of least elasticity parallel to the vertical axis of the plate. If then a dark hyperbola is seen to traverse the field, the mineral is negative.

II. *Biaxial Minerals*.—In order to render intelligible the directions later given, there is here stated the method published in the text-books for determining the positive or negative character of a biaxial mineral plate.

If a line of extinction of a biaxial plate properly cut is placed parallel to one of the cross-hairs, it shows a cross with unequal arms; but if the line of extinction makes an angle of  $45^\circ$  with that cross-hair, it shows two dark hyperbolas, whose vertices or eyes mark the position of the vertical axes. Accompanying the cross and hyperbolas are coloured lemniscate figures. Oftentimes the hyperbolas are wanting, and only the coloured lemniscata can be seen; but by the insertion of the quartz wedge, the hyperbolas can frequently be brought into the field.

(a) The positive or negative character of this biaxial plate can then be determined by placing the plate on the stage in such a position that a line joining the hyperbola eyes, or bisecting the lemniscata through their longest direction, shall form an angle of  $45^\circ$  with the cross-hairs. Push in the quartz wedge with its axis of least elasticity parallel to the line joining the hyperbola eyes. If the hyperbola eyes open and move toward the centre of the lemniscate figure, the mineral is positive.

Push in the quartz wedge with its axis of least elasticity perpendicular to the line joining the hyperbola eyes. If these eyes open and move toward the centre of the lemniscate figure, the mineral is negative.

Of course, if in either case the eyes contract and move outwards, this is proof, when the axis of least elasticity of the quartz wedge is perpendicular to the line joining the hyperbola, that the mineral plate is positive; but if they move outward when the axis of elasticity is parallel to the chosen line, the mineral plate is negative.

This method is less satisfactory in practice than the one where the eyes open and move inwards.

(b) The above method given in our text-books can be supplemented by one that can be employed in numerous cases when both of the hyperbola eyes cannot be seen, but only one of them or only the lemniscate arcs. In either of these cases the positive or negative character of the mineral plate can be ascertained; if one can determine the position of the line joining the hyperbola eyes or optic axes, by the form of the interference figures, by the position of the larger arm of the cross, or by any other means. When this direction is observed, place the arcs so that the direction of the line joining the hyperbola vertices shall be perpendicular to, or shall bisect them; also have this line make an angle



of  $45^\circ$  with the cross-hairs, as before. Push in the wedge with its axis of least elasticity perpendicular to the arcs, or parallel to the line joining the hyperbola eyes. If the lemniscate arcs move in towards the centre of the field with their convex side forwards, the mineral is positive.

Push in the wedge with its axis of least elasticity tangent to the arcs, or perpendicular to the line joining the vertices. If the arcs then move in with their convex side forwards, the mineral is negative. If the arcs move outwards with their concave side forwards, the mineral in the first position of the wedge is negative, and in the second position positive.

(c) If the distance between the hyperbola eyes is not so great but that they lie within the field of view, the mica and gypsum plates can both be employed to determine the positive and negative characters when the lemniscate figure is placed as before, with the line joining the hyperbola eyes forming an angle of  $45^\circ$  with the cross-hairs of the eyepiece. Insert either the mica plate with its axis of least elasticity parallel to the chosen line, or else insert the gypsum plate with its axis of least elasticity perpendicular to the chosen line. With either plate in this position, the arcs on one side of the hyperbola eyes will enlarge and those on the other side contract. If the arcs that lie on the inside of the eyes, or nearest the centre of the figure, enlarge, and those on the outside contract, the mineral is positive. On the other hand, if the arcs nearest the centre contract, and the outside arcs expand, the mineral is negative. This method can be used with plates that have too great an axial divergence for their determination when the unsymmetrical cross is placed with its arms parallel to the cross-hairs."

**Microscopical Water Analysis.\***—Herr C. Mez's introduction to the examination of water deals principally with drinking water and sewage. The first part treats of the vegetable and animal organisms ordinarily found in impure water, and the second and larger portion of the work deals with the methods of microscopical analysis.

One of the chief merits of the work consists in an attempt to introduce a simplified nomenclature: thus, *Diplococcus flavus liquefaciens tardus*, *Micrococcus pyogenes albus*, *Bacterium fluorescens liquefaciens minutissimum*, are designated *M. flavovirens*, *M. pyoalbus*, *B. minutissimum*.

**Cleaning Slides intended for Water-stuck Sections.†**—M. J. G. de Groot declares that the following procedure is absolutely perfect for cleaning slides intended to be used for sections which are to be stuck on by the water method. On a clean cloth is spread a little common chalk, and the cloth having been moistened with a drop of water, the slide is rubbed therewith. The slide is again rubbed with a clean cloth moistened with water, and is then ready for use.

\* Biol. Centralbl., xviii. (1898) pp. 507-9. Hedwigia, xxxvii. (1898) pp. 96-7.

† Zeitschr. f. wiss. Mikr., xv. (1898) pp. 62-3.

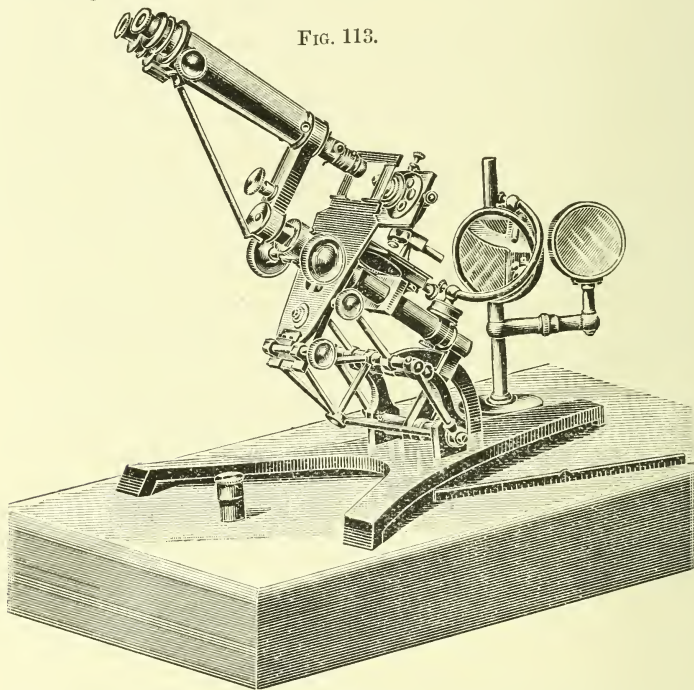
## MICROSCOPY.

## A. Instruments, Accessories, &amp;c.\*

## (1) Stands.

**Large Binocular Microscope designed and made by an Amateur.**—At the meeting of the Society on November 16th, the President brought under the notice of the Society a large binocular Microscope (fig. 113) which was not only designed, but actually made by an amateur, and accompanied an exhibition of a photograph of the instrument with the following remarks.

FIG. 113.



“The three largest Microscopes that have been constructed since the introduction of achromatism, with which I am acquainted, are first, Tulley’s, 1826.† The second was made by G. Lowden, jun., of Dundee, in 1851.‡ This was 4 ft. long, had a body-tube 4 in. diameter, and weighed  $1\frac{1}{2}$  cwt. The third was made by C. D. Ahrens, and was exhibited before this Society in 1888. It had a body-tube of  $4\frac{1}{2}$  in. diameter, and it stood  $2\frac{1}{4}$  ft. high.§

“Of these three the second can hardly be called a practical construction, and the third was intended solely for polariscope work; on the other hand, the one represented in the photograph, although large, is a practical and serviceable working instrument. A one-foot rule is placed

\* This subdivision contains (1) Stands; (2) Eye-pieces and Objectives; (3) Illuminating and other Apparatus; (4) Photomicrography; (5) Microscopical Optics and Manipulation; (6) Miscellaneous. † Carpenter, 7th edition, fig. 112, p. 147.

‡ Journ. R.M.S., 1882, p. 851, fig. 156.

§ Op. cit., 1889, p. 273, fig. 39.

on the table beside it, forming a rough scale by which its size may be estimated. The action of the various parts of this Microscope is so unlike that of any other instrument that they will hardly be understood from an inspection of the photograph; a detailed account is therefore necessary. To the flat tripod foot, which is about  $18 \times 12$  in., are fixed two lateral flat upright posts, braced together at their upper end by a fixed horizontal rod; to the centre of this rod one end of a quadrant is attached, its other end being fixed to the base.

"The quadrant and its lower point of fixture are clearly seen in the photograph; but the fixed horizontal rod, which supports the upper end of the quadrant, is hidden by another horizontal bar; its terminal screw, which passes through the right-hand vertical upright post, can however be seen. Through the bases of the upright posts is fixed a stout cylindrical horizontal bar, its centre being the centre of the quadrant. A strong arm, forming a radius of the quadrant, turns on the middle of this bar; its upper end, which embraces the quadrant, is produced a short distance beyond the quadrant, and forms a rigid attachment for the lower end of the tail-piece. To the extremities of this lower horizontal bar is pivoted the lower end of a lattice bracing, the upper end of this bracing being attached to the lower fixed stage-plate, which in this Microscope is behind the tail-piece. The plan of this lattice-work is clearly seen in the photograph; a more minute description is therefore unnecessary. Attention however may be drawn to the third horizontal rod, to which are attached the stays and struts of the lattice bracing, and which moves with the lattice bracing when the Microscope is inclined. The Microscope can be clamped in any inclined position by a screw, which, passing through the upper end of the radius, pinches the quadrant. Thus far we have an entirely original method of supporting a large Microscope, which ensures rigidity, apart from undue weight. Passing on to the Microscope itself, we see that it is constructed on the Ross model, with an unusually long (for that model) fine adjustment lever. The coarse adjustment is of the ordinary type, racking a prism bar out of the tail-piece. The body, which is binocular and 12 in. long, is supported at the back by a strut.

"In this part we meet with little or no divergence from known forms; but in the stage we find considerable novelty. The mechanism is as follows:—there are three plates, the upper one, which is identical with the actual stage of the Microscope, is attached by a pivot, seen a short distance from its lower end, to a middle plate, which carries a tangent screw. These two plates slide in a vertical dovetailed groove in the third or lowest plate, the vertical movement being actuated by rack and pinion in the usual way. The transverse movement is a transverse movement in arc; this is performed by the tangent screw at the lower end of the middle plate causing the upper plate to revolve on the pivot seen a little above it. Special notice should be given to this stage with regard to its rigidity, which depends of course upon the rigidity of its lowest plate; but this, as we have seen, is supported by no less than five struts of the lattice bracing and by the tail-piece. The result obtained by this design is an open, thin, and unhampered stage, and I know not where you will find its equal in this respect.

"From the stage we pass on to the substage, in which we shall find some more novel and interesting details. The substage itself has the usual rectangular movements for centering, and it is also fitted with a slow



motion focussing screw, the milled head of which is seen at the lowest point of its support, immediately in front of the tail-piece. This support of the substage swings in arc (the reflection of a portion of the arc can be seen in the mirror), the centre of rotation being the object on the stage. The substage, carrying the condenser, can also be further turned obliquely to the optic axis by means of the pivot in its front support. By means of these two motions extreme obliquity of illumination can be obtained.

"With regard to the invention of the swinging substage, there is one figured in Adams on the Microscope, 1798 (Pl. 9, fig. 5), in connection with Jones's Lucernal Microscope, as improved by the Rev. John Prince, of Massachusetts, and Mr. John Hill, of Norfolk.\* The idea underlying this construction was not for purposes of oblique illumination, as we now understand it, i. e. for sending an oblique beam below the stage on a transparent object, because this kind of illumination would be simply useless with a lucernal Microscope which projected the image on a ground-glass screen. But the swing was applied in order that the substage might be turned round to the upper side of the stage, so that it might become a super-stage illuminator for opaque objects. Substage oblique illumination was neither intended nor used at this date. The next time we meet with a swinging substage is in Grubb's Sector Microscope † (described before the Royal Irish Academy on May 10th, 1852, and patented 1854). In this Microscope the substage, with a right-angled prism attached beneath it, was fixed to a slot, which traversed in a radial groove.‡ Coming to more recent times (1871), Tolles, § in America, revived the swinging substage, to facilitate the resolution of lined tests with oblique light. He was followed by Bulloch || (1873) and Zentmayer ¶ (1876) in America, as well as by all the principal makers in this country, Powell excepted. This absurd craze culminated in the Ross-Wenham radial Microscope of 1882.\*\* I am, however, happy to say that the 3/4 axial cone has slain the Lernæan Hydra, and both swinging substages and radial Microscopes have sunk to rise no more. Returning, however, to the model before us, we have a very perfect and early form of swinging substage; for I first saw this Microscope in 1870, and at that time it must have been about ten or fifteen years old. Several of the objectives used with this Microscope were computed and made by its designer."

\* Journ. R.M.S., vol. vii. ser. 2, 1887, p. 297, fig. 45.

† Op. cit., vol. iii. 1880, p. 1056, fig. 126, and Proc. R. Irish Acad., v. (1853) p. 296.

‡ The following is abstracted from a footnote which occurs in a paper on 'The Measurement of Aperture,' written by the learned and Reverend J. K. Robinson, D.D., Dean of Armagh, with reference to a new Microscope Mr. Grubb had made for him (Quarterly Journal of Microscopical Science, iii. p. 166, 1855).

"This Microscope (*Grubb's Sector*) deserves to be known. . . . Mr. Grubb's illuminator is a prism whose aberrations are corrected for a lamp placed at a given distance in the plane of the stage. It travels on a graduated arc of 120°, and through this range its focus continues on the object. . . . If raised above the stage it gives at once a capital illumination for opaque objects; it acts well with Lieberkühn and Nicol's prism, and trifling additions make it equally effective with Mr. Bergin's parallel illuminator, which shows some objects with peculiar distinctness." Mr. Bergin's illuminator was a combination of a rhombic prism with Mr. Shadbolt's parabolic reflector.

§ Journ. R.M.S., vol. iii. 1880, p. 1061, figs. 128, 129.

|| Tom. cit., 1880, p. 1068, fig. 133.

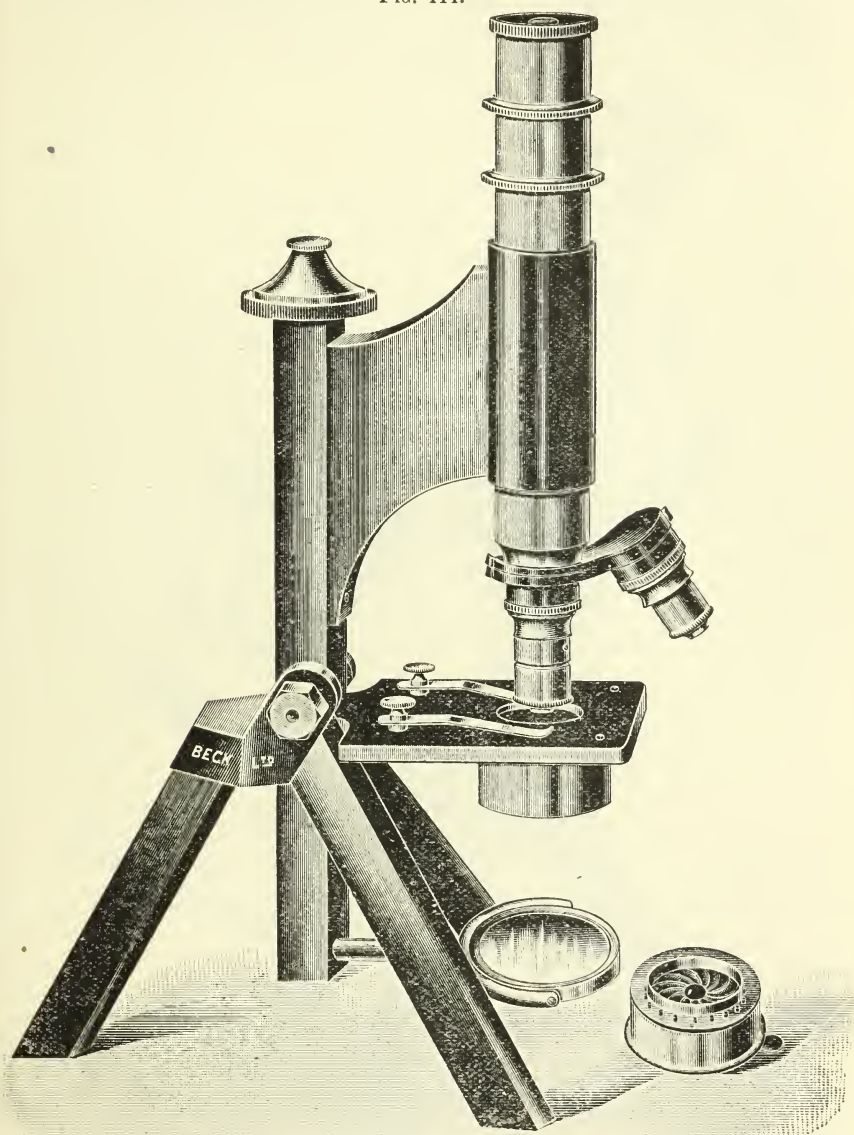
¶ Op. cit., vol. ii. 1879, p. 320, and 2 figs.

\*\* Op. cit., vol. ii. ser. 2, 1882, p. 256, 4 figs. and pl. p. 145.



"British Students'" Microscopes.—This name is given by Messrs. R. and J. Beck to their new forms of Students' Microscopes, two of which

FIG. 114.

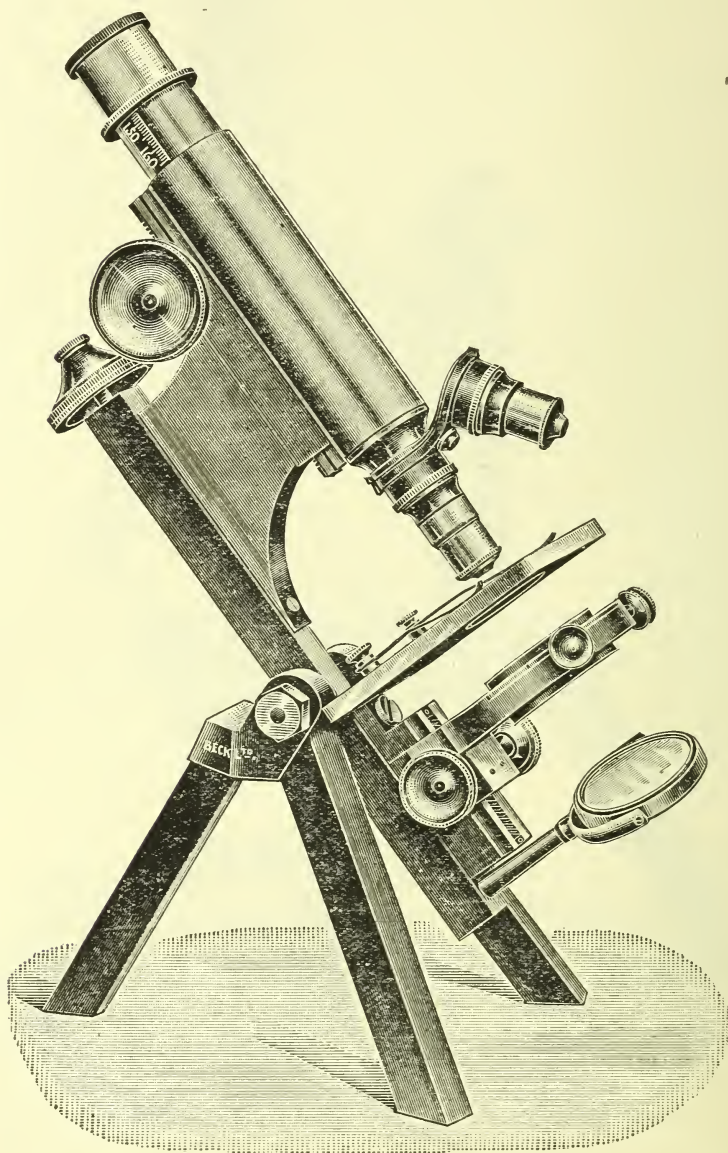


are here figured. By making large numbers of these instruments, and by the extensive use of machinery in their production, Messrs. Beck  
1898 2 z

consider that they have brought out an excellent class of low-priced Microscope.

Fig. 114, which shows the cheapest of the set, has a sliding coarse-adjustment. Its stand is a solid heavy tripod, with a spread of 6 in.

FIG. 115.



between each foot. It has a joint for inclination, and the fine-adjustment is regulated by a micrometer-screw and large milled head. The large square stage has a distance of  $2\frac{1}{2}$  in. from centre of stage to limb, and allows a very large culture-plate to be examined. The under-stage is provided with a fitting of the full size, and is furnished with an improved form of iris-diaphragm enlarged or contracted by means of a handle—shown in the figure. Each instrument is provided with a plane and concave mirror. An Abbe form of condenser at once converts the instrument into a very efficient Microscope for bacteriology and other high-power study.

Fig. 115 shows a more expensive form of the same instrument. The difference is partly in the coarse adjustment, which here is a spiral rack and pinion. The draw-tube is engraved in millimetres, and at once records the exact amount of mechanical tube-length in use. Another addition is a rack and pinion focussing and screw centering substage; the milled head of the focussing substage pinion is extended beyond the legs of the tripod, thus placing the focussing adjustment of the substage always within easy reach, even when the instrument is used in a vertical position. By these means all the requirements for the very highest power work are secured, and the very best condensers and highest power object-glasses can be successfully manipulated.

**"Fram" Microscope.**—The "Fram" Microscope, recently brought out by Messrs. W. Watson and Sons, is illustrated in fig. 116. The object of this design is to produce a Microscope of the highest class at a very moderate price. The working parts are fitted and finished by hand, so as to obtain the smoothest and most precise action. The foot is of the tripod pattern with a spread of 7 in., and the instrument is absolutely steady in any working position. The coarse adjustment is Watson's diagonal rack and pinion, and the fine adjustment is the lever pattern applied to all the instruments manufactured by the firm, which has been tested for many years, and found satisfactory. One revolution of the milled head moves the body  $1/300$  in., thus giving very exact adjustment for high-power work, while the compensating screws provide a remedy for any slackness that may arise after a long period of use.

The coarse and fine adjustments are of the pattern adopted by Messrs. Watson in all their Microscopes. The stage, which is large, is of the Nelson horse-shoe shape, and the fittings are of the universal size throughout. The body with the draw-tube closed measures less than 6 in., and with the draw-tube extended 10 in.; it is therefore permissible to use objectives corrected for either the Continental or English tube-lengths. The lower end of the draw-tube is provided with the universal thread. In its plainest form the Microscope is provided with the ordinary tube fitting beneath the stage, to carry the condenser, &c., and is arranged to be turned aside from the optical axis when not required. This can be replaced by a compound substage, fitted with rack and pinion focussing and screw centering adjustments. A sliding bar having a new form of spring fitting, affording a very smooth motion, has been designed for the instrument. The objectives are so mounted as to be approximately in focus when revolved on a nose-piece, and the eye-pieces are all arranged to work in the same focal plane.



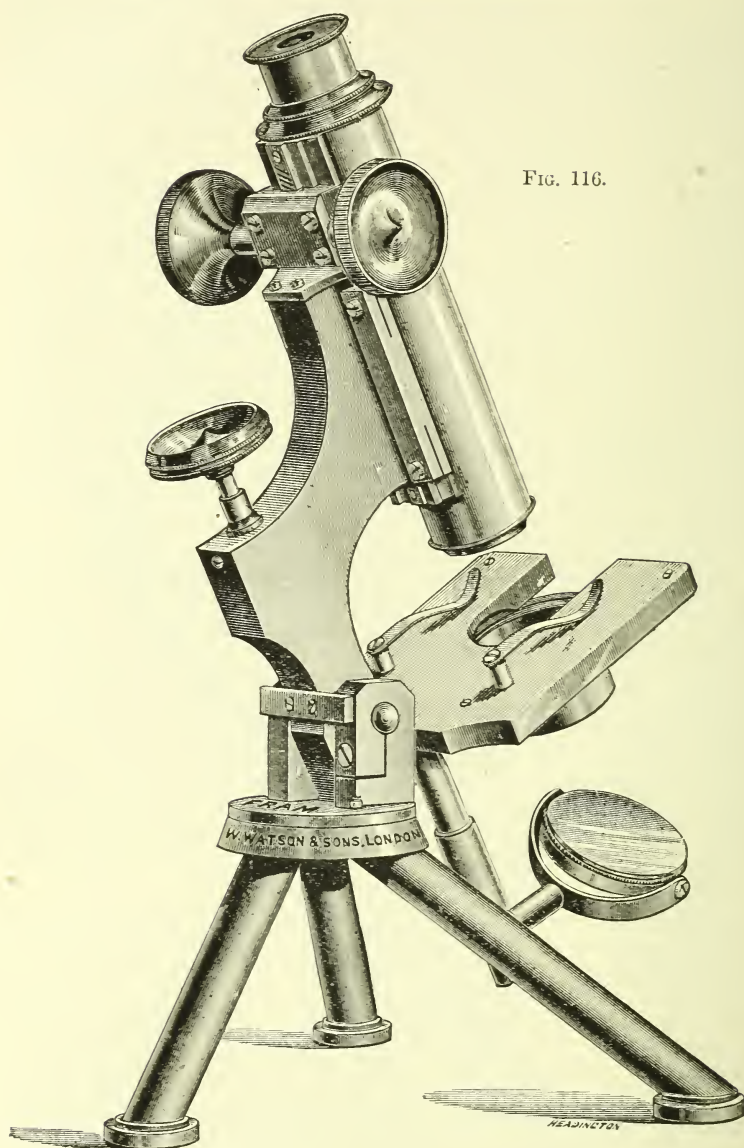


FIG. 116.

**Old French Microscope.**—The President exhibited, at the meeting of the Society on October 14th, an old French Microscope, which, if its date could be accurately determined, might be of considerable interest. The body has an archaic look about it, reminding one of Joblot's (1718), but the rack and pinion focussing to the stage prevents its date

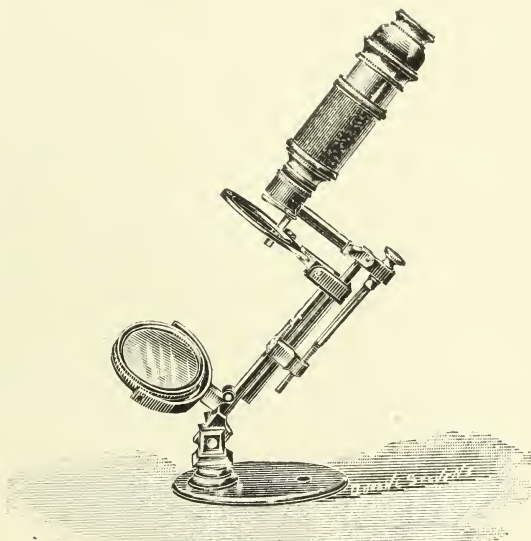


being put earlier than about 1765. Below the stage is a short piece of conical tube, and below that is a wheel of graduated diaphragms. This conical tube is a survival of the black ivory cone, mentioned by Henry Baker in his description of Culpeper and Scarlet's Microscope in 1743.

An interesting point is that there is no known instance of a wheel of graduated diaphragms between the years 1702 and the establishment of achromatism 1825-30; so that if the date of this Microscope could be established prior to 1825, it would be the earliest example of the reintroduction of the wheel of graduated diaphragms since 1702.

**Microscope by John Cuff.**—The President exhibited an interesting old Microscope by J. Cuff of Fleet Street (fig. 117), which in its day

FIG. 117.



was called "an improvement on Ellis's Aquatic Microscope" (1755). It differs, however, from that instrument in seven points.

(1) A compound body having a single eye-lens (no field-lens) can be attached.

(2) It is provided with a fine adjustment of the John Marshall type (lens and not stage focussing).

(3) The stage has lateral movement on a pivot.

(4) The instrument is inclinable.

(5) The pillar is mounted excentrically on its oval base-plate, and it is capable of rotation, which gives the Microscope greater stability in different positions.

(6) It can be folded up for portability.

(7) A clip is provided to clamp the slide to the stage, and this is

the earliest known example of a slide-clip, as distinguished from the spring, plates, &c., in vogue at that time.

This model has played an important part in the evolution of the Microscope; for it was copied in 1771 by Adams, who added a rack and pinion to the forward movement of the body over the stage. It was also the type copied by Raspail for his Microscope, which seventy years later was very popular in France.

*Notes.*—(a) The lateral movement of the stage may be considered as a modification of the stage of Benjamin Martin's "Universal Microscope" (1740).

(b) The forward movement of the body over the stage is effected by a *sprung* slide.

(c) The manner of clamping the slide to the stage is the same as that still employed by Powell in his iron Microscope, and is the prototype of the spring-clip at present so largely used on Continental Microscopes.

(d) This instrument is the earliest known example of a pillar, mounted excentrically, and rotating on its foot to increase the stability. This idea was copied by George Adams in 1771. It was reinvented by Mr. A. McLaren.\* It has also lately been introduced by Messrs. Ross & Co.† It ought to be pointed out that the pillar of John Marshall's Microscope, 1704, was also mounted excentrically, and was capable of rotation, inasmuch as it had a ball-and-socket joint at the bottom. This, however, was not meant to increase the stability, but to swing the Microscope clear of its base, which was necessary for purposes of illumination, there being no mirror. Stability in John Marshall's Microscope was obtained by fixing lead to the bottom of the box.

Speaking to-day with our fuller knowledge of the essential points of a Microscope, we should severely criticise Cuff's Microscope. The attachment of the stage to the pillar by a pivot cannot be commended, neither can the hinged joint on the limb which carries the body. Both these devices deprive the instrument of all steadiness, and render it fit for work with the lowest powers only. The designer evidently thought that the transverse motion of an object was better secured by a pivoted stage than by the pushing of a slider between two plates compressed by a stiff spring; in this he was probably right, for we must compare the instrument with those in use at that time, and not with our modern stages. The hinged joint in the limb was to allow the Microscope to be placed in a horizontal position, so that any object held in the stage forceps might be examined with a Lieberkuhn with direct illumination, which was a method much in vogue at that time. In brief, it was a throw back on what has been termed "the telescope mount."

The probable date of this instrument, which is signed, is *circa* 1760.

## (2) Eye-pieces and Objectives.

**Improved Huyghens Eye-piece.**‡—Dr. Hugo Schroeder begins a discussion of the principle of this eye-piece by pointing out that, as is well

\* Journ. R.M.S., 1884, ser. ii. vol. iv. fig. 9, p. 111.

† Journ. R.M.S., 1894, pp. 507-8, figs. 39 and 40.

‡ Central-Ztg. f. Opt. u. Mech., No. 10, May 15, 1898, pp. 91-3.

known, it fulfils the conditions of achromatism, viz. that  $\Delta = \frac{P+p}{2}$ ,

where the letters have their usual meanings. It is clear that  $P$  and  $p$  may have an infinite number of separate values, and may yet give the same value for  $\Delta$ . Which of these infinite values are the best? For the last century it has been usual to put  $P = 3p$ , and "Huyghens' ocular" usually has this construction. Dr. Schroeder admits that in this form the combination is not ill adapted for high magnifications, but considers it ill suited for weak magnifications, and the nearer  $\Delta$  approaches the value unity the worse the combination is. The necessary insertion of a stop which excludes half the field of view is a grave defect. He has therefore recalculated the equations, and after several years' experience gives a table for the decimal values of  $P$  from 1 to 3, from which the following lines are extracted.

The conditions underlying the table presuppose (1) that both lenses are plano-convex; (2) that they are of the same material; (3) that in the first approximation the various coloured images are brought to the same size; (4) that the eye-point is exactly at the half-focus of the eye-glass, in order that the observer should not come too near the lens.

$F$  = Objective focus.

$P$  = Field-lens focus.

$p$  = Focus of the eye-lens.

$\Delta$  = Distance of both lenses with respect to their cardinal points.

$s$  = Distance of field-lens from the objective.

$E$  = Equivalent focus of the ocular.

$L$  = Length of the instrument from the first to the last lens.

$m$  = Magnification of the instrument.

$d$  = Distance of diaphragm from the field-lens.

$D$  = Distance of objective image from the field-lens.

$f$  = Focal length of the primary rays after quitting the field-lens measured therefrom.

$\delta$  = Diameter of the diaphragm in the focus of  $p$ .

$\Theta$  = Half the visible field of view.

$P$	$p$	$\Delta$	$s$	$F$	$m$	$d$	$D$	$E$	$f$	$L$	$2\Theta$	$\delta$ max.
1.0	1	1.00	2.00	2	2	0	0	1	2.00	3.00	$28^{\circ} 58'$	0.500
1.5	1	1.25	4.50	4.80	4.00	0.25	0.300	1.200	2.25	5.75	38 56	0.667
2.0	1	1.50	10.00	10.67	8.00	0.50	0.667	1.333	2.50	11.50	47 10	0.800
2.5	1	1.75	27.50	28.57	20.00	0.75	1.071	1.429	2.75	29.25	54 4	0.909
3.0	1	2.00	$\infty$	$\infty$	$\infty$	1.00	1.500	1.500	3.00	$\infty$	60 0	1.000

**New Microscope Objective for Zoological and other Biological Investigations under Water.\***—Dr. H. Hartwig, of Jena, describes the above apparatus, which has been made to his designs by the Zeiss firm.

\* Zeitschr. f. wiss. Mikr., xv. (1898) p. 162.

He considers that Zeiss' water-immersion system D\* is usually the best to use for the observation of living creatures in water-chambers. In many such cases, however, the desideratum is not so much a lens of powerful resolution and high magnification as of extended field of view and deep penetration. His new objective is constructed to meet this want, and he names it the "Planktonsucher."

The Planktonsucher has a front focus of 35 mm., a working distance of 36 mm., and a numerical aperture of 0.11. For a tube-length of 160 mm. and Zeiss' Huyghens' oculars 1 to 5, the following values are obtained for the diameter of the objective field and for the magnification:—

Huyghens Ocular.	Objective Field. mm.	Magnification.
1	3.5	25
2	3.3	35
2*	4.2	35
3	2.4	50
4	2.0	60
5	1.7	80

In consequence of the use of the new Jena glasses, which after many years' experience have proved extraordinarily durable, a very good correction-position could be given to the system. The image is completely plane and free from astigmatism quite close up to the periphery, even when ocular 2\* with its extended view-field is used, so that adjustment affects the whole view-field uniformly. As the interference of the ray-pencils is almost apochromatic, the images display a clean-cut distinctness. In order to be able to carry on observations in water, the lenses are fastened on the end of a cylindrical nickelled brass tube, so that entry of the water is impossible.

In order to secure small creatures on the floor of the water-chamber, three narrow glass bars are cemented on to the floor, and a cover-glass placed on them (below the water-level), so that the objects are enclosed in a thin stratum, which stratum can be explored without altering the adjustment of the objective.

### (3) Illuminating and other Apparatus.

"Newtonian" Universal Science Lantern (Ives and Newton's Patent).—This lantern has been devised with a view to producing an instrument which shall be extremely portable and which shall yet be capable of producing, with a minimum of trouble and rearrangement, most of the experiments required by science lecturers. As shown in the illustration (fig. 118), it is fitted with lantern-slide front, microscopic attachment, and spectroscopic front. The lantern is so fitted that any one of the three fronts can be exchanged for the other by simply sliding the front from the centre to the one side or the other as may be required.

The first front is for showing the ordinary slides or diagrams, and comprises a 4-in. double condenser and 8-in. best double achromatic front lens, exhibiting slides as well and clearly as any lantern that is made.

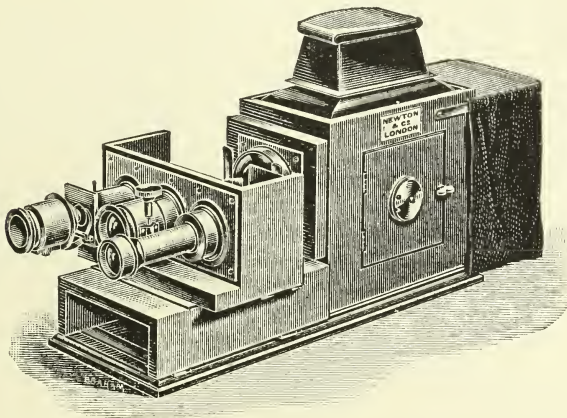
The second front is a Microscope attachment, and is of more interest to the readers of this Journal, perhaps, than the other parts of the instru-



ment, and is fitted with a movable substage condenser and low-power objective. The adjusting substage is so made that a spot-lens or dark-ground illuminator can be used, also a Lieberkuhn for illuminating opaque objects. A large Nicol can be used for showing crystals; rock sections by means of polarised light show well on the screen. The case with which the Microscope can be shifted to the lantern front for showing photomicrographs makes the instrument especially useful to the lecturer.

The third front is for spectroscopic work, and is fitted with slit, direct-vision prism, and achromatic focussing lens, which project a brilliant spectrum on the screen without the necessity of placing the lantern at an angle. A comparison prism is also fitted, by which at the same time an image is projected on the screen of any medium of which the absorption spectrum is being shown.

FIG. 118.



The whole of this part of the apparatus can be lifted off, leaving a clear base-board, so that a vertical attachment, or photochromoscope, elbow polariscope, or any front can be substituted that may be wanted directly. The condenser is mounted on a hinge, and can be removed or put into position instantly. Either lime-light or the electric arc can be used in this lantern; and as it is made throughout in Messrs. Newton & Co.'s workshop, it is perhaps unnecessary to say that every detail has been carefully arranged, and that the workmanship is of a high class.

#### (6) Miscellaneous.

**Abbe's Theory of the Microscope.\***—Karl Strehl, of Erlangen, propounds the seven following propositions, which seem to him to be of fundamental importance for rightly understanding Abbe's theory of the Microscope.

“(1) Of the continuations of the plane-wave which falls perpendicularly on the primary optic axis and illuminates the object, only the direct continuation is a plane-wave, the deflected ones are by their very nature not plane-waves.

\* Central-Ztg. f. Opt. u. Mech., No. 18, pp. 71-2 (2 figs.).

"(2) The diffraction-spectra are, according to Abbe (in monochromatic light), set up as the real images generated by the system, and due to the bright parts of the virtual diffracted appearances generated by the objective structure in the image-forming infinitely distant planes.

"(3) The stereometric place for equality of phase is not so much the image-forming focal plane, as a spherical surface concentric to the image-point and generated by the image-forming focal point.

"(4) The direct primary maximum lies at the image-forming focus, but the deflected secondary maxima do not lie in the image-forming focal planes; the collective diffraction-spectra are not aplanatic images.

"(5) The diffraction-spectra do not lie generally on a spherical surface, and even if they do, then this spherical plane is generally not concentric to the image-focus; generally the ray-paths have not equal distances between the diffraction-spectra and the image-focus, i.e. the diffraction-spectra are not in the same phase.

"(6) If the objective is aplanatic, then the locus of the real diffraction-spectra is formed with unequal phase by means of a spherical surface concentric to the image-focus; consequently it is easily understood that the normal microscopic image is erected at a definite point of the optical primary axis (i.e. at the centre of the spherical surface), and not in any desired point, so that the diffraction-spectra lie with equal phase in the focal plane.

"(7) When the surface (generated by the image-forming focus) is of such a kind that it contains the collective diffraction-spectra in the same phase, I call it the 'wave-surface' (*Wellenfläche*). This 'wave-surface' is constant for the aplanatic arrangement of the system."

## B. Technique.\*

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

**Artichoke as Nutrient Medium.**†—M. M. Roger recommends artichoke as a nutrient medium, as many bacteria and yeasts grown on this substratum exhibit a characteristic appearance. With some species the infection-site assumes a green hue, with others it remains uncoloured. *B. subtilis* forms a deposit in 24 hours, the subjacent substratum being coloured green. Anthrax does the same in 3-4 days. Typhoid has no action on the substratum. *B. prodigiosus* when incubated imparted the green staining, though the red pigment did not form. At room temperature the cultures of *B. prodigiosus* exhibited the red colour, while the green staining was absent.

The author suggests that the green pigment is due to the oxidation of some constituent of the artichoke owing to the (vital?) action of the microbes. That it is an oxidation process is obvious from the fact that when oxygen is excluded, the green pigment is not formed. The pigment is soluble in water, insoluble in alcohol, chloroform, and ether. Acids turn it red, while bases restore the green colour.

\* 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.

† C.R. Soc. Biol., v. (1898) pp. 769-71.

## (2) Preparing Objects.

**Preparation and Fixing of Algæ.\***—Prof. F. Oltmanns recommends the following process for the Floridææ. The algæ are placed in chromic-potassium-sublimate-glacial-acetic-acid, or in Rath's picrin-osmium-platinum-chloride-acetic-acid, and then, after a short time, in 70 per cent. alcohol, until they no longer impart to it a yellow colour. Rath's mixture fixes the chromatophores well. The preparation may then be stained by hæmalum or by very dilute solution of hæmatoxylin (at 60° C.), or by Heidenhain's hæmatoxylin-iron-alum. Carmin does not answer so well.

**Isolating Ganglion-cells and Unstripped Muscle-fibres.†**—According to Herr J. Arnold, ganglion-cells and unstripped muscle-fibres are easily isolated by the iodo-potassic iodide method. The objects may be placed for 2-3 days in 10 per cent. potassium iodide solution, and then for a similar time in iodopotassic iodide solution (5 drops of the strong solution to 10 ccm. of 10 per cent. potassium iodide solution), or the latter mixture may be employed from the start. In the first case the cells are better isolated, in the second they preserve their form more perfectly.

It is a very good thing to add a few drops of an aqueous solution of eosin to the isolating fluid.

**Methods for Demonstrating Myxosporidia.‡**—Herr F. Doflein used for his investigations on Myxosporidia material derived from sea- and fresh-water fish. It is indispensably necessary that living and fresh material should be studied, as the study of the finer morphology of the spores is a matter of extreme difficulty in clarifying media. The best fixative was found to be Flemming's solution, though sublimate, picro-acetic acid, and picro-sulphuric acid can be used with advantage, especially as after the latter reagents carmin stains can be employed; for, as is well known, after Flemming's solution these stains are not extremely successful. In order to obtain preparations of the forms inhabiting the urinary and gall-bladders, the following method was adopted. A drop of the fluid in which Myxosporidia were suspended was spread on a slide and fixed with one of the before-mentioned fluids. If the bile would not coagulate a little blood was added. In this way a crowd of Myxosporidia are fixed in a thin film, and can be further treated as a section. After fixation in Flemming's solution, the best pigments were safranin and gentian-violet, and the hæmatoxylin-iron. After the other fixatives, borax-carmin, Mayer's carmin, hæmatoxylin, hæmalum, hæmatoxylin-eosin, or orange G, Bismarck-brown, and methylen-green were useful occasionally. For showing up the walls and margins of cells, indulin is a useful accessory to iron hæmatoxylin.

**Method for Demonstrating the Shape of Spaces and Passages in Embryos.§**—Herr F. Hochstetter communicates an ingenious method which he devised for showing the changes occurring during the development of the membranous labyrinth. This method, with modification and

\* Bot. Ztg., lvi. (1898) 1<sup>te</sup> Abth., p. 100.

† Arch. f. Mikr. Anat., lii. (1898) pp. 762-73 (1 pl.). See Zeitschr. f. wiss. Mikr., xv. (1898) p. 226.

‡ Zool. Jahrb. (Abth. f. Anat. u. Ontog.), xi. (1898) pp. 281-350 (7 pls. and 20 figs.). See Zeitschr. f. wiss. Mikr., xv. (1898) pp. 217-8.

§ Zeitschr. f. wiss. Mikr., xv. (1898) pp. 186-92 (1 pl.).



improvement, was applied to embryo lungs for the purpose of demonstrating the shape of the air-passages and sacculæ. The preparations are immersed for a short time in a mixture of two parts chloroform and one part oil of cloves. They are then removed, and dried on filter-paper. When the preparation begins to turn white (less than 5 minutes), it is transferred to oil of cloves. The spaces in the labyrinth are now filled with air or a mixture of air and chloroform vapour, and when examined by reflected light look as if they were filled with quicksilver. Though the picture may last long enough to take a drawing or a photograph, it is a transient one, owing to the absorption of the air by the oil of cloves. More lasting preparations were obtained from embryo lungs. In this case a solution of finely powdered Indian ink was used. The solution was sucked in through the trachea as the air disappeared from the lung, and in this way beautiful preparations of the shape of the air-passages and air-sacs were obtained.

The photographs given of lung preparations of the embryos of rabbits and fowl are excellent demonstration objects.

**Removal of Pigment from Zoological Specimens.\***—Dr. R. Jander recommends Fol's decalcifying solution for removing pigment from zoological specimens, such as mantle of Lamellibranchs, leeches, Arthropods' eyes, fish-skins, &c.

The solution consists of one per cent. chromic acid, 70 vols.; nitric acid, 3 vols.; water, 200 vols.

The pigment is removed in from 12 to 48 hours, according to the bulk of the object. The solution may be used for sections as well as for whole objects. Sections must be stuck on by means of some adhesive, such as very thin albumen.

The solution has the further advantage of fixing and decolorising simultaneously. The acids are easily removed by means of water.

**Hardening Blood, Sputum, &c., on Slides.†**—Dr. C. Ritter prefers slides to cover-glasses for making preparations of morbid and other fluids, such as blood, pus, sputum, urine. The fluid should be poured on, and the slides placed in glass capsules or vessels containing the fixative, such as osmic acid or formalin. The slides are made to rest on fillets or blocks with the film side downwards, so as to expose the surface to the action of the reagent.

**Demonstrating Medullated Nerve-fibres.‡**—Herr A. E. Smirnow demonstrates the medullated nerve-fibres in the molecular layer of the cerebellum by the Weigert-Pal and Golgi methods. The whole cerebellum of a freshly killed dog is placed in a mixture of 4 parts of 5 per cent. bichromate of potash and 1 part of formol for 1–8 weeks. The organ is then cut in half and immersed in 3–5 per cent. bichromate of potash solution, which is renewed daily for a week. After an immersion of from 2–5 weeks, one-half is treated by the Weigert-Pal method, and the other cut up into pieces of 1–2 cm. in length.

These pieces are placed in the following mixture:—Five per cent. bichromate of potash, 5 vols.; 2 per cent. osmic acid, 1 vol. After

\* Zeitschr. f. wiss. Mikr., xv. (1898) pp. 163–5.

† Tom. cit., pp. 159–61 (2 figs.).

‡ Arch. f. Mikr. Anat., lii. (1898) pp. 195–202 (1 pl.). See Zeitschr. f. wiss. Mikr., xv. (1898) pp. 246–7.



1-1½ weeks, the pieces are transferred to a weak aqueous solution of nitrate of silver, and then for 48 hours or longer to a 1 per cent. solution thereof.

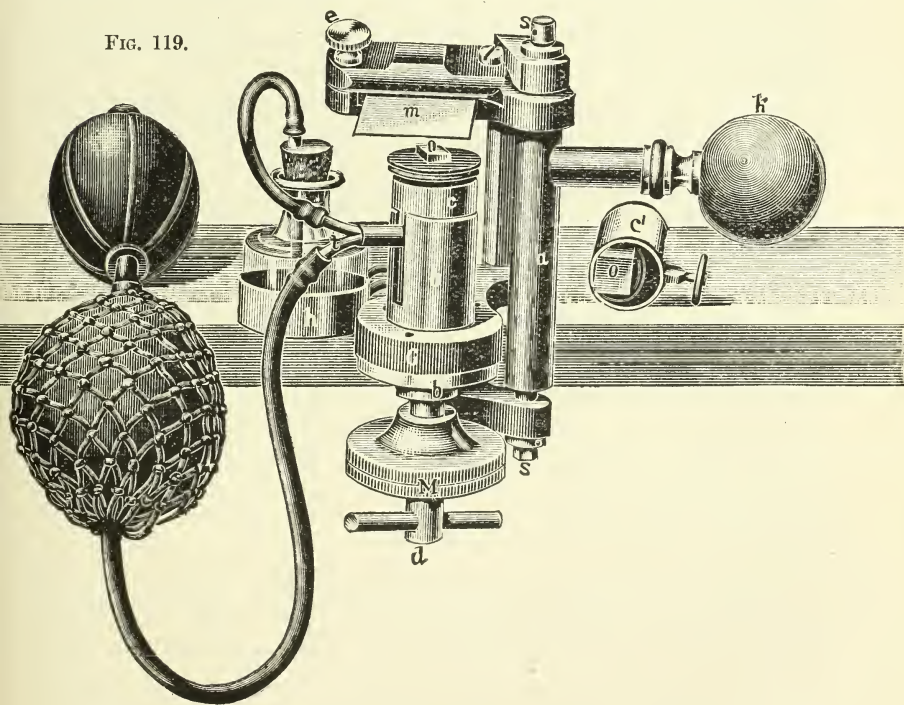
This modification of Golgi's method gave good results also with human cerebella or cerebra which had been kept for 2-3 days in a cool place.

**Preparation of Diphtheritic Toxin without Meat.\***—Prof. Spronck, having found that yeast favoured the production of the diphtheritic toxin, now prepares the toxin by the following method. Ordinary yeast, not brewer's yeast, is used. One kilo. is boiled in 5 litres of water for 20 minutes, the yeast being constantly stirred with a spatula. The decoction is poured out and allowed to stand 24 hours. To the supernatant fluid are then added 5 grm. of salt and 20 grm. of pepton. The fluid, which is slightly acid, is neutralised with soda, 7 ccm. of normal soda solution being added to the litre. It is then hot-filtered through paper, distributed into vessels, and sterilised at 120°. Thus prepared, the diphtheritic toxin is twenty times stronger than that prepared from old meat.

(3) Cutting, including Imbedding and Microtomes.

**New Microtome.†**—M. P. Francotte describes a microtome made by Jung which has the following advantages (fig. 119). It is of

FIG. 119.



\* Ann. Inst. Pasteur, xii. (1898) pp. 701-4.

† Bull. Soc. Belge de Microscopie, xxiv. (1897-98) pp. 18-21 (1 fig.).

moderate price. It is adapted for cutting objects imbedded in celloidin or in paraffin, or by the ether-freezing method. It is constructed of iron, the greater part of the surface being nickelled and the rest varnished. It is clamped to the table by a large binding-screw *d*, and if required to cut under water or alcohol, it is fixed to a board placed vertically.

The microtome proper is of the Ranvier type. The micrometer-screw is moved automatically by an excentric which is capable of adapting itself to one or more divisions of a graduated circle *b*. The requisite number of divisions is determined by the operator, each corresponding to a micron when the handle *k* is pushed forward. The excentric is set by merely turning a ring, and thus gearing the microtome for 1 to 36 microns.

The knife-carrier is attached to the vertical piece *a*, and moves on the pivots *ss* when the handle *k* is pushed backwards or forwards.

The illustration (fig. 119) shows the apparatus as arranged for ether-freezing.

#### (4) Staining and Injecting.

**Weigert Methods of Staining.\***—Prof. C. J. Herrick reports a series of experiments with the Weigert staining methods in studying the components of the cranial nerves in bony fishes, which rests largely on the myelination of the nerves. The results are given for the different fixing reagents, mordants, &c., and form a body of valuable suggestions for those who propose applying these methods to the lower Vertebrates.

**Fixing and Staining Starch-grains.†**—The results described on p. 639 were obtained by Herr J. H. Salter by the use of the following processes of fixing and staining.

Fleming's mixture was found, on the whole, to be the best fixing material. Sublimate-alcohol and picrin-alcohol also gave good results when the staining reagent used was acid-fuchsin.

For staining the plastid in which the starch-grains are imbedded, acid-fuchsin and iron-hæmatoxylin gave much the best results, especially when the fixing material used was sublimate; 20 grm. of acid-fuchsin were used in 100 ccm. of anilin-water. The starch-grains themselves may be stained by methyl-violet or gentian-violet in aqueous solution, both showing the stratification fairly well, especially if the section be first treated with a concentrated aqueous solution of orange G. For a double stain the material may be fixed by sublimate and stained by acid-fuchsin followed by methyl- or gentian-violet, the excess of violet being then removed by orange. The chloroplasts and leucoplasts, as well as the whole of the cytoplasm, take up the fuchsin, while the starch-grains absorb the violet.

**Sudan iii., a Selective Stain for Fat.‡**—Dr. A. G. Nicholls confirms the observation of Rieder, who found that Sudan iii. is useful for histological work, especially for differentiating fat. A saturated solution of Sudan iii. in 96 per cent. alcohol is, after filtration, diluted two-thirds

\* N. York State Hospital's Bull., Oct. 1897. See Amer. Nat., xxxii. (1898) p. 802. † Jahrb. f. wiss. Bot. (Pfeffer u. Strasburger), xxxii. (1898) pp. 118-21.

‡ Microscopical Bulletin, xv. (1898) p. 31.

with 50 per cent. alcohol, and filtered again. Sections, after a few minutes' immersion, are washed in 60-70 per cent. alcohol, drained, and mounted in glycerin or Farrants' medium. Fat is stained a carmin-red, but the colour is a golden-yellow if the particles be small.

**Microchemical Staining of Cell-walls.\***—M. J. Chalon records the results of a new series of experiments made on the following vegetable tissues:—pine, willow, *Cordyline*, mistletoe, *Dracæna*, agave, hemp, cotton, rose, maize. All the material was first treated with eau de javelle to destroy the starch and cell-contents, the cell-wall being the only part experimented on. The single stains used were, hæmatoxylin, benzo-azurine, magdala-red, benzo-purpurin, naphthol-black, coralline, methylen-blue, orseille.

The double stains were, campeachy and fuchsin, fuchsin and methylen-blue; alum-carmin and methylen-blue or iodine-green; carmin, campeachy and methyl-green; prussian-blue and safranin; campeachy and benzo-purpurin, anilin-blue and magenta.

These double stains were tried on palm, iris, pineapple, ketmie, maize, *Cordyline*, mistletoe, willow.

Further experiments and the conclusions are promised later.

**New and Rapid Method for Double Staining Blood.†**—Dr. R. Garcia fixes and stains blood in the following way. A drop of blood and a drop of sterilised bouillon are mixed together on a cover-glass. When the mixture is dry, the cover-glass is placed on a slide and the film fixed by heating the slide over a flame. This takes scarcely a minute. For staining the author employs eosin and methylen-blue in simple solution; the stains are used successively, the superfluous fluid being washed off each time with water, after which the preparation is mounted in balsam. The whole process takes about 5 minutes.

It is preferable to use the eosin before the methylen-blue; and in order to make the bouillon more durable, a few drops of formol may be added.

**Neutral Red for Staining Hæmoglobigenous Granules.‡**—Dr. E. Giglio-Tos demonstrates the hæmoglobigenous granules in the erythrocytes of certain animals—lamprey, fowl embryo, frog, guinea-pig, &c.—by means of a saturated solution of neutral red in 0·8 per cent. sodium chloride.

A drop of the staining solution and a drop of the blood are mixed together on a slide, and a cover-glass imposed. In 5-10 minutes the preparation may be examined.

**Modification of Van Ermengem's Method of Staining Flagella.§**—Dr. J. W. W. Stephens has obtained beautiful results from a modification of Van Ermengem's method,|| which consists in using "largin," one of the many compounds of silver and albumen, instead of nitrate of silver. The usual procedure is followed, the silver bath being a 2 per cent. largin solution which contains about 0·2 per cent. of silver. The film may be passed from silver to gallic acid three or four times, or oftener.

\* Bull. Soc. Roy. Bot. Belgique, xxxvii. (1898) pt. ii. pp. 12-29.

† *Crónica Médica-Quir. Habana*, xxiii. No. 23. See *Zeitschr. f. wiss. Mikr.*, xv. (1898) pp. 236-7.

‡ *Zeitschr. f. wiss. Mikr.*, xv. (1898) pp. 166-71.

§ *Lancet*, 1898, ii. p. 874.

|| Cf. this Journal, 1894, p. 405.



The film thus passes through (1) largin, from 2 to 10 minutes; (2) gallic acid, 2 to 10 minutes; (3) largin, till clear; (4) gallic acid again if necessary, and so on.

The cover-glasses should always be burnt on a piece of wire gauze to remove all fat.

By this method clean well-stained preparations can be obtained with ease and certainty.

**Staining Intestinal Canal by Van Gieson's Method.\***—Dr. W. Möller recommends Van Gieson's method for staining sections of intestinal canal. The method was originally intended for the central and peripheral nervous system, and consists in staining first with hæmatoxylin, and afterwards with a mixture of acid fuchsin and picric acid. The author adopts the following modified procedure. (1) Stain for half an hour in Delafield's hæmatoxylin. (2) Immerse in distilled water for 12–24 hours. (3) Stain for 1/2–1 minute in Van Gieson's mixture, which is composed of 150 ccm. of a saturated aqueous solution of picric acid, and 3 ccm. of a saturated aqueous solution of acid fuchsin. (4) Wash in distilled water (1/4–1/2 minute). (5) Dehydrate in 90–96 per cent. alcohol (2–5 minutes). (6) Transfer to absolute alcohol for 1 minute. (7) *Origanum* oil; (8) Canada balsam. To the water (4) and the spirit (5) must be added a few drops of Van Gieson's solution to prevent the too great extraction of the picric acid.

The material dealt with had been kept in spirit for years, and the sections were "celloidin-sections."

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

**New Method for Fixing Paraffin Sections to the Slide.†**—Herr K. Koninski's gelatin-formalin method is based on the fact that formalin renders gelatin firm and insoluble. The slides are covered with a thin film of gelatin, after the manner of photographers. When the gelatin has set the plate is ready. Upon the dry plate are arranged the ribands of sections, and having been smoothed down by the aid of warm water, any excess of water is poured off. The plate is then warmed until the gelatin is liquefied. The superfluous gelatin is removed with bibulous paper, and the plate allowed to dry. When dry it is placed in pure formalin for about 10 minutes. Thus fixed the gelatin film holds the sections so firmly that the slide may be immersed in boiling water without fear.

#### (6) Miscellaneous.

**Microscopic Detection of Phosphorus-containing Compounds.‡**—Prof. A. B. Macallum discusses the difficulties connected with the microscopic detection and localisation of compounds containing phosphorus, and especially the value of the reducing agents hitherto employed after the use of ammonium molybdate for the purpose. He finds that pyrogallol is unreliable, and recommends the use of a recently prepared aqueous solution of phenylhydrazin hydrochloride of 1–4 per cent. strength. This, in the absence of alcohol or a caustic alkali, gives a green colour where phospho-molybdate compounds are present, but only

\* Zeitschr. f. wiss. Mikr., xv. (1898) pp. 172–7. † *Tom. cit.*, pp. 161–3.

‡ Proc. Roy. Soc. Lond., lxiii. (1898) pp. 467–74.



a faint yellow reaction where ammonium molybdate alone is present; the great advantage of this reducing agent being that it is not necessary, as in the case of pyrogallol, to remove uncombined ammonium molybdate before subjecting the tissues to the reducing process. The method is as follows:—The tissues, either fresh or hardened in alcohol, are subjected, for periods varying from 10 minutes to 48 hours, to the action of a nitric-molybdate solution made by dissolving one part by weight of pure molybdic acid in four parts by weight of strong ammonia, and adding thereto slowly fifteen parts by weight of nitric acid sp. gr. 1·2. The reagent acts best at a temperature of 35° C., and the action is progressive; the inorganic phosphates are first affected, then the lecithin, and finally the organic phosphorus. When the reaction is completed, the tissues are exposed to the phenylhydrazin hydrochloride solution for a minute or two, washed in distilled water, dehydrated, cleared in oil of cedar, and mounted in balsam. The lecithin may be removed from the tissues before the phosphorus test is applied by extracting with ether and then subjecting to prolonged (five hours) treatment with boiling ethyl-alcohol in a Soxhlet apparatus. The inorganic phosphorus may be partially removed by treating the tissues with 20 per cent. acetic acid; but it is possible, by examining the preparations after the molybdate solution has acted for about 10 minutes, to determine the relative amount of inorganic phosphorus present; an increase of the reaction after the first 10 minutes demonstrates the presence of inorganic phosphorus.

**Microchemical Demonstration of Alkaloids.\***—Herr H. Barth, in an exhaustive article on the microchemical demonstration of alkaloids, states that alkaloids are present in some part of the fruit or seed, from the husk to the endosperm and embryo.

The reagents employed for determining the presence of the alkaloids were those ordinarily used in pharmaceutical and toxicological chemistry. These reagents were chiefly precipitants, and those which gave some characteristic colour. In a few instances the reagents gave better results when used as vapour than in solution.

The results of the author's observations appear to show that the functions of alkaloids are various, some being essentially excretory and protective, others, especially such as exist in the endosperm and embryo, are reserve substances and nutritive.

**New Method for making Casts.†**—Dr. G. A. Peters has invented a method for making casts by which the minutest details of the object are rendered visible. The process consists in spraying melted paraffin over the whole surface, at the same time cooling it by spraying ice-cold water, strengthening the mould with plaster of Paris, and removing it in as many sections as may be required. The mould is made of hard paraffin. The surface of an object requires no preparation unless covered with hair. It is in this case oiled, and covered by means of a spray with a cream composed of cornflour, glycerin, and alcohol. The paraffin is sprayed on through a special ejecting apparatus, which is practically nothing more than a spray surrounded by a hot-water jacket. The

\* Bot. Centralbl., lxxv. (1898) pp. 225-31, 261-7, 292-300, 326-44, 369-78, 401-9, pl.).

† Brit. Med. Journ., 1898, ii. pp. 621-4 (5 figs.).

mould is then strengthened by a backing of plaster of Paris, and having been divided up into sections in the usual way by means of threads, is removed.

Though the procedure is somewhat cumbersome, the results are extraordinarily good.

**Lee and Mayer's Outlines of Microscopical Technique for Zoologists and Anatomists.\***—The general scope of Mr. N. Bolles Lee's work on microscopical technique is too well known to English microbiologists to require any description.

Herr P. Mayer, of Naples, has recently brought out a German edition which, while purporting to be a translation, is really a revision. The work has been much improved by the alteration and the care bestowed on it.

**Cement for Glass.†**—The following mixture is recommended for cementing glass used in aquaria. Gum elastic is dissolved in benzin till the fluid has a syrupy consistence. White lead and linseed oil varnish are rubbed up to a paste and mixed with the gum solution. The cement may also be used for sticking glass to wood.

**Bone-Corrosion Preparations.‡**—Dr. Stanislaus von Stein describes a new method of obtaining models in caoutchouc of the inner ear. The first necessity is that the temporal bone should be thoroughly cleaned; to effect this it may be injected with hot concentrated soda solution. When clean it is to be injected with "Rosa-Kautschuk" dissolved in chloroform, and placed for twenty-four hours in a beaker filled with solution which is allowed to slowly thicken. The bone is then reinjected if necessary, allowed to dry completely, and the caoutchouc scraped from the surface except over the openings. The next process is that of vulcanisation, accomplished by placing the bone in a cuvette in a thin plaster of calcium sulphate, closing the cuvette tightly, and placing it for an hour and a half in a vulcanising apparatus. It is then taken out of the cuvette, the adherent sulphate removed in water, and the preparation decalcified by being placed in a solution containing hydrochloric acid in the proportion of 1 to 5. The perfectly hard preparation is then washed, dried, and varnished. The advantages of the method are claimed to be its rapidity and cheapness, and the permanence and excellence of the result.

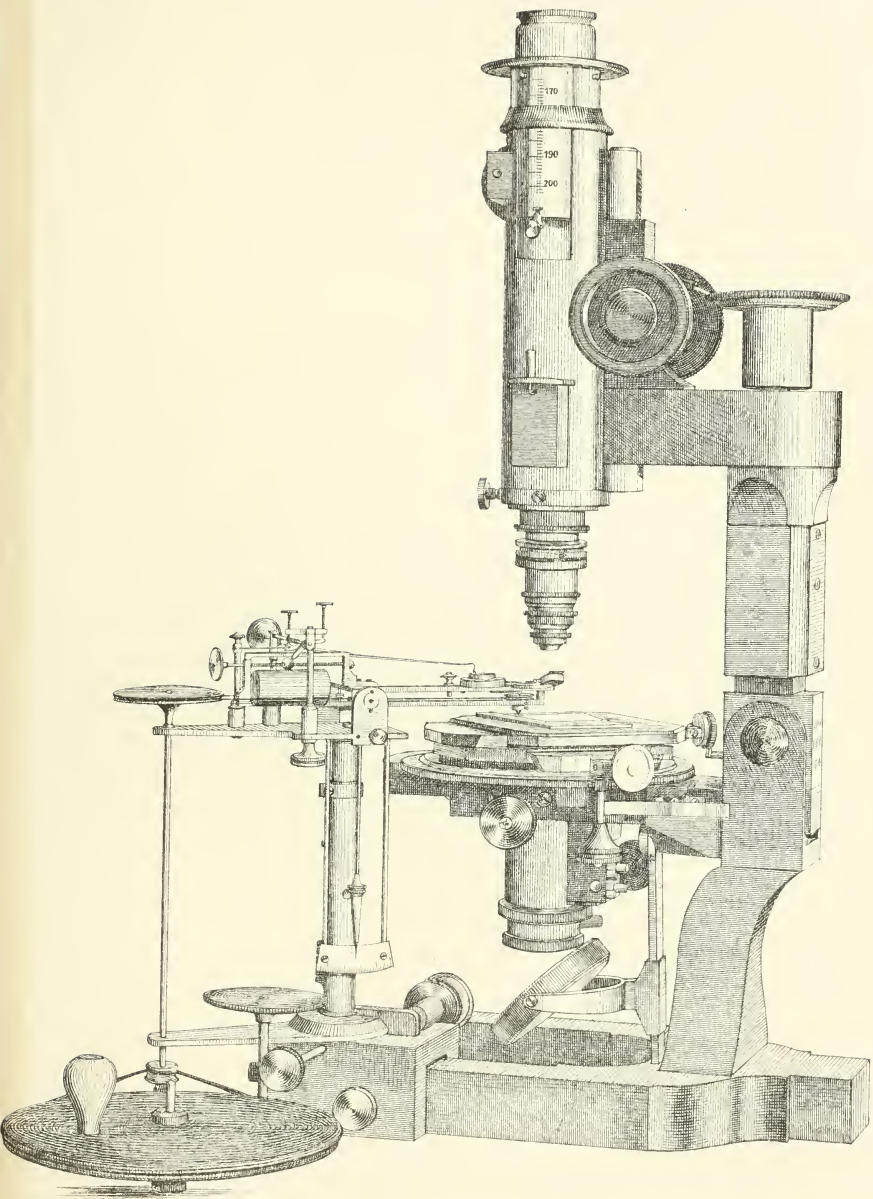
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As a Frontispiece to the current volume, we give a reproduction of an excellent photograph, taken by Mr. Washington Teasdale, which may interest our older Fellows, of the room in King's College, where the meetings of the Society were held from the year 1867 until we moved to the present rooms in Hanover Square in 1890.

\* Berlin, 1898 (Friedländer u. Sohn). See Bot. Ztg., lvi. (1898) 2<sup>te</sup> Abth., pp. 185-6.

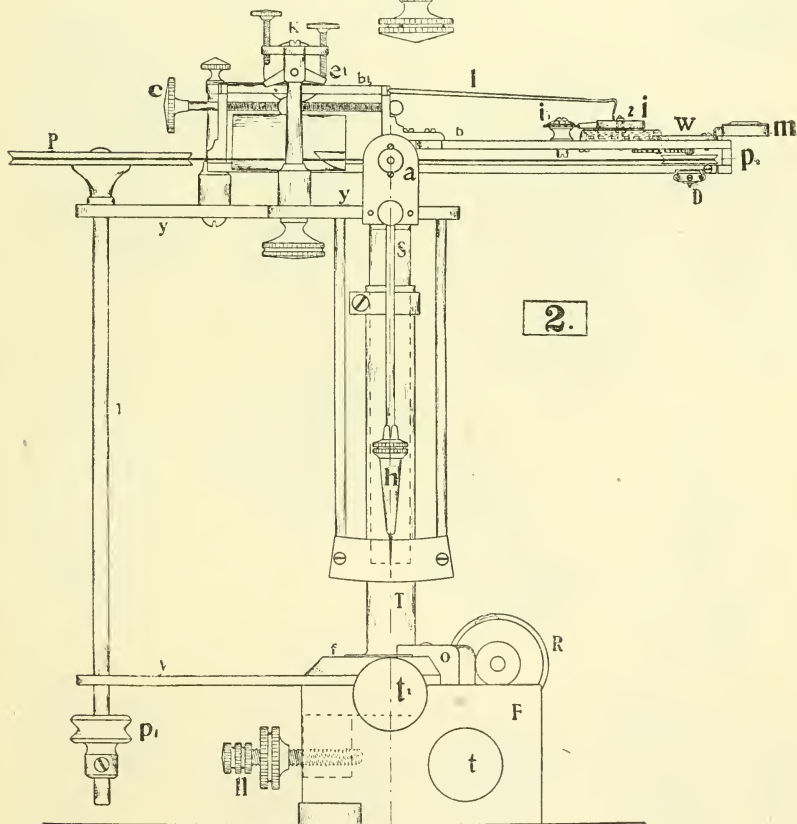
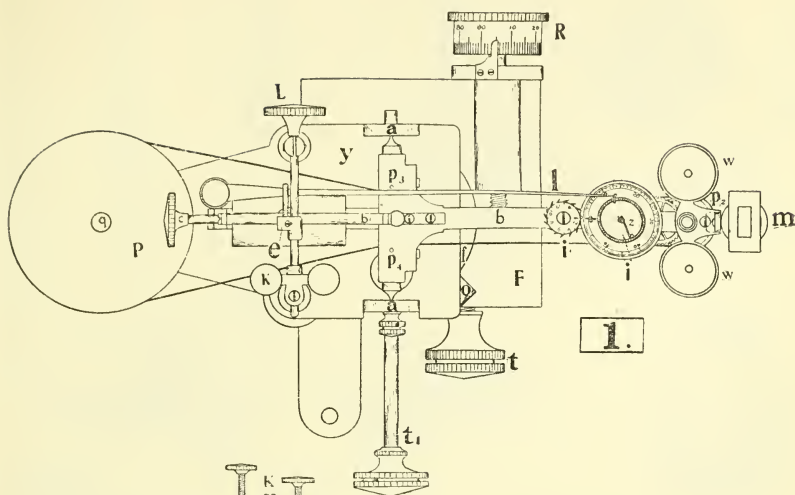
† Zeitschr. f. angew. Mikr., iv. (1898) p. 109.

‡ Anat. Anzeig., xv. (1898) pp. 112-6.



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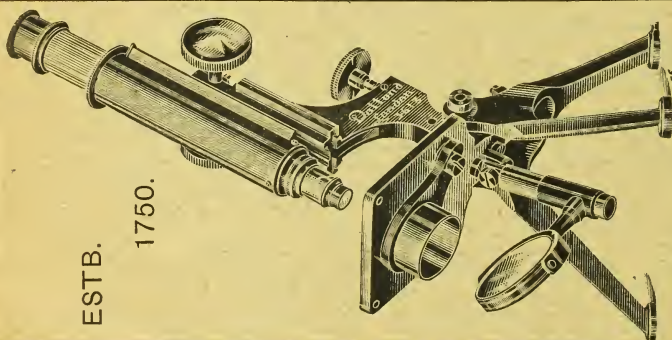
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