Directions for making and preserving microscopical preparations / by Harting of Utrecht.

DIRECTIONS FOR MAKING AND PRESERVING MICROSCOPICAL PREPARATIONS.

BY HARTING OF UTRECHT.

[FROM THE MONTHLY JOURNAL OF MEDICAL SCIENCE FOR APRIL 1852.]

[The following directions are translated, in a slightly abridged form, from different parts of Harting's work on the microscope.[1] They have been selected as likely to prove useful to that now numerous class of students who prosecute original researches with the aid of the microscope. Personal experience enables us to attest the value of some of these hints; and the fact that Professor Harting's unrivalled cabinet of microscopic preparations, comprising more than 6000 specimens put up with his own hands, is indebted for its completeness and preservation to the methods of manipulation here described, is sufficient evidence of their excellence.—*Trans.*]

Very few objects can be preserved unaltered when dry, and even when this is possible, as in the case of hairs, fish-scales, and the like, the method is not to be in general recommended. Such objects, when surrounded by air, possess too little transparency to permit a satisfactory definition of their component parts. It is only for preserving the scales of insects and certain *test objects* that the dry method is useful, and even preferable, from the superior distinctness with which it enables the observer to make out the different sorts of lines upon these bodies. The simplest mode of mounting these scales for microscopical examination, is to lay a few of them upon an ordinary glass object-slide, which may be moistened with the breath, if this is found necessary to make the objects adhere to it. A glass covering-plate, of suitable thickness, is then laid upon the object; and finally there is pasted round both slide and cover a piece of paper, having in its centre an opening corresponding to the position of the object.

Different specimens from the organic kingdom would, if simply put up in the dry way, speedily become the prey of vegetable and animal parasites. This is the case, for instance, with sections of organs like the lungs, preserved by inflation and subsequent drying. To prevent this disadvantage, I am in the habit

[1] Het Mikroskoop, deszelfs gebruik, geschiedenis en tegenwoordige toestand. Utrecht. 3 vols. 1848-50.

EDINBURGH: SUTHERLAND AND KNOX.

of moistening such preparations with oil of turpentine, which, on evaporating, leaves upon the surface a very delicate varnish-like coating, which suffices for its protection.

Most microscopical objects, however, require to be mounted in some fluid, the nature of which must be varied according to the prôperties of the substance which it is wished to preserve. The fluids which I employ are the following:—

I.—*Saturated Solution of Chloride of Calcium.*

1st, This solution, which must be perfectly free from traces of iron, is of very general utility, and may be employed in all cases in which the substance to be preserved is of moderate firmness or hardness. In this solution all preparations of bones and teeth, sections of hairs, feathers, fish-scales, whalebone, &c., are best preserved. It may be also used with advantage for mounting specimens of many minute animalcules provided with a hard integument, such as cheese-mites, the itch-insect, small fresh-water crustacea, and the like. It is likewise the best preservative for vegetable preparations, whose cell-walls or vessels have undergone a partial incrustation, and is also very useful for displaying the shells or *loricæ* of the siliceous bacillariæ and diatomaceæ.

In using it, one only requires to lay the object on a slide, and to moisten it with a drop of the solution, taking care, at the same time, to remove the air-bells which may be formed here and there. Two pieces of paper, corresponding to the thickness of the object, are next pasted to the extremities of the slide, and the whole is then covered with a second glass plate of the same size. If it should now be found that too little fluid has been applied to the object, or that part of it has run off, a drop of the solution may be applied to the edge of the slide, and will find its way between the glasses by capillary attraction. A piece of thin paper may be inserted between the glasses, to promote the flow of the fluid towards the preparation, or to rectify the position of the object when it has become displaced.

For attaching the strips of paper to the glass slides in this and other cases, the best material that can be used is starch paste, with which a little arsenious acid is mixed, in order to prevent the formation of a species of mould which is otherwise apt to gather round the preparations.—Vol. ii., p. 347-350.

Of late I have discovered a fault in this mode of mounting preparations. In many which have been preserved in drops of the chloride of calcium solution, there have formed numerous branches of a species of Hygrocrocis, which spread from preparation to preparation, and from box to box, threatening totally to destroy all specimens which have been put up in this way. I have consequently discontinued the practice of mounting specimens in chloride of calcium solution, to which the air still has access; and when I now employ this or any other fluid, am careful to exclude the influence of the atmosphere by touching the edges of the covering-plate with a cement which I have elsewhere described (see p. 376). This procedure has the additional advantage of not requiring the use of a saturated solution: it may be diluted, in proportion to the delicacy of the specimen, with from two to ten parts of water.—Vol. iii., p. 470.

TABLE FOR MUTUAL CONVERSION OF BRITISH AND FOREIGN LINEAL MEASUREMENTS.

* The following table, constructed on the same principles as the tables of modern works on Quantitative Chemical Analysis, will greatly facilitate the comparison of Foreign and British measurements, especially when minute, and involving decimal fractions.

To convert—	1	2	3	4	5	6	7	8	9	
1. British Inches into Millimetres,.....	25·39954	50·79908	76·19862	101·5982	126·9977	152·3972	177·7968	203·1963	228·5959	Millimetres.
2. Do. Old Paris Lines,.	11·25936	22·51872	33·77808	45·03744	56·29680	67·55616	78·81552	90·07488	101·33424	Paris Lines.
3. Do. Rhineland or Prussian Lines,	11·65275	23·30550	34·95824	46·61099	58·26374	69·91649	81·56923	93·22198	104·87473	Prussian Lines.
4. Millimetres into British Inches,.....	·03937079	·07874158	·11811237	·15748316	·19685395	·23622474	·27559553	·31496632	·35433711	British Inches.
5. Do. Old Paris Lines,...	·44329	·88658	1·32987	1·77316	2·21645	2·65974	3·10303	3·54632	3·98961	Paris Lines.
6. Do. Rhineland or Prussian Lines,	·45878	·91756	1·37633	1·83511	2·29389	2·75267	3·21145	3·67022	4·12900	Prussian Lines.
7. Old Paris Lines into British Inches,	·088815	·177630	·266445	·355260	·444075	·532890	·621705	·710520	·799335	British Inches.
8. Do. Millimetres,.....	2·25586	4·51172	6·76758	9·02344	11·27930	13·53516	15·79102	18·04688	20·30274	Millimetres.
9. Do. Rhineland or Prussian Lines,	1·03494	2·06988	3·10482	4·13976	5·17469	6·20963	7·24457	8·27951	9·31445	Prussian Lines.
10. Rhineland or Prussian Lines into British Inches,	·085817	·171633	·25745	·343267	·429083	·51490	·600717	·686532	·77235	British Inches.
11. Do. Millimetres,.....	2·179704	4·359408	6·539112	8·718816	10·89852	13·07822	15·25793	17·43763	19·61734	Millimetres.
12. Do. Old Paris Lines,	·9662407	1·9324814	2·8987221	3·8649628	4·8312034	5·7974441	6·7636848	7·7299255	8·6961662	Paris Lines.

ILLUSTRATIONS OF USE OF THE ABOVE TABLE.

I.—Example.

Given 245·9003 Paris Lines. Required the value in British Inches.

By line 7 of Table,—

Old Paris Lines.		British Inches.
200	=	17·7630
+ 40	=	3·55260
+ 5	=	·444075
+ ·9	=	·0799335
+ ·0003	=	·0000266445
		21·8396351445 British Inches.

II.—Example.

Given ·00215 Millimetres. Required the value in British Inches.

By line 4 of Table,—

Millimetres.		British Inches.
·002	=	·00007874158
+ ·0001	=	·000003937079
+ ·00005	=	·0000019685395
		·0000846471985 British Inches.

III.—Example.

Where extreme exactitude is not required, only one or two decimal places need be used. Thus,—

Given 21·8396 British Inches. Required the value in Paris Lines.

By line 2 of Table,—

British Inches.		Paris Lines.
20	=	225·19
+ 1	=	11·26
+ ·8	=	9·01
+ ·04	=	·45
		245·91 Paris Lines *very nearly*.

Data, from which the Table has been calculated, extracted from Mr Woolhouse's Table, in the *Encyc. Metropolitana*,—British foot = 1. Metre = 3·2808992. Old Paris foot = 1·06578. Rhineland or Prussian foot = 1·0298.

W. R.

II.—*Canada Balsam.*

Many varieties of this substance are met with in commerce, differing from each other in purity and colour. The best, which is alone suitable for the use of the microscopist, is perfectly transparent, almost colourless, and very viscid. Canada balsam is used as a preservative material in all cases when it is of importance to heighten the transparency of the object,—as in mounting specimens of pollen grains, sections of hard fruit envelopes, corals, shells, and especially injected preparations of organs which suffer no change from previous drying (see March Number, pp. 245-253). It is also employed for mounting many powder-like mineral substances,—mud, containing diatomaceæ; chalk, with foraminifera, &c. Specimens of this last sort should be spread with water on the object-slide, which should then be warmed till the powder is dry. When cold, the specimen is covered with the balsam, which, if too viscid, may be brought to the consistence of syrup by the addition of a little oil of turpentine. The mixture with water may be dispensed with in the case of most other objects; but it is generally expedient to moisten them first with oil of turpentine, in order to get rid of air-bubbles which may be present, and finally to cover them with the balsam. The pasting and covering of the slides are performed just as when the chloride of calcium solution is used.

III.—*Creozote Solution.*

This fluid may be prepared either by distillation with water, or by filtering a saturated solution of creozote in one part of alcohol of s. g. 867°, after mixing it with twenty parts of water. It is useful for all preparations of muscle, cellular tissue, tendon, ligament, cartilage, sections of bones and teeth which have been treated with acid, the fibres of the crystalline lens, etc. For the preservation of adipose tissue, of the ultimate nerve tubes, and of the blood-corpuscles, it is not well adapted. Objects put up in it, after a certain time, usually acquire a brownish-yellow tint.

IV.—*Solution of Arsenious Acid.*

To prepare this solution an excess of arsenious acid is boiled with water, which is then filtered and diluted with thrice as much water. This fluid is one of the most suitable preservatives for preparations from the animal kingdom; all the tissues mentioned under the last head, and also the adipose tissue, may be kept unaltered in it; and as they acquire no yellow colour, or a far slighter tinge, during their immersion, I have of late years accorded a general preference to the arsenical over the creozote solution.

V.—*Solution of Corrosive Sublimate.*

This is prepared by dissolving one part of corrosive muriate of mercury in from 200 to 500 parts of water. The strength of the solution must be varied according to the nature of the object to be preserved; hence it is well, when the required degree of concentration is not ascertained, to put up several preparations with solutions of different strengths. This procedure is especially ap-

plicable to blood-corpuscles, which can be preserved unaltered in no other fluid with which I have experimented. Thus the blood-corpuscles of the frog require a fluid containing $\frac{1}{400}$th of corrosive muriate; those of birds a solution of $\frac{1}{300}$th; those of mammalia and man $\frac{1}{200}$th.

These solutions are likewise useful for keeping the elementary parts of the brain, spinal cord, and retina, although all these structures, in whatever fluid they are put up, undergo some alteration. Cartilage, and the fibres of the crystalline lens, keep well in these fluids; but other fibrous tissues lose too much of their transparency when in contact with them. They may be used, however, for preserving muscular fibre, whose cross markings they render more distinct.

For preparations of delicate vegetable tissues, and, in general, of all tender organs in which it is desired to retain the starch globules and chlorophyl unaltered, for fresh water algæ, diatomaceæ, confervæ, infusoria belonging to the division rotifera, &c., a solution containing $\frac{1}{400}$th or $\frac{1}{500}$th of corrosive sublimate is the best preservative with which I am acquainted.

VI.—*Solution of Carbonate of Potass.*

This may be made of various strengths, with one part of the salt dissolved in from 200 to 500 parts of water, and is the best material for preserving the primitive nerve tubes. Other fibrous tissues may be kept tolerably well in it, but become more transparent than in the fresh condition. This is sometimes advantageous, as, for example, when we wish to display the respiratory apparatus of insects with the ramifications of the air-tubes.

VII.—*Solution of Arsenite of Potass.*

I have, in a few instances, made use of a solution of arsenite of potass in 160 parts of water, to preserve the primitive nerve-tubes. It has been found as effectual as the carbonate of potass solution.

In employing the chloride of calcium solution[1] and Canada Balsam, it is unnecessary to take measures to prevent the evaporation of the fluid. The first remains always fluid,—chloride of calcium being a deliquescent salt; and as the outer surface of the balsam hardens, the escape of the liquid portion is prevented.

But it is otherwise with the last-mentioned preservative fluids (Nos. III. to VII.) To prevent their evaporation, it is necessary to employ a cement or luting to prevent air from having access to the fluid. Different compositions have been recommended for this purpose; but I have found none more serviceable than that employed by gilders to make gold-leaf adhere to mirror and picture frames. The following is the receipt for the preparation of this so-called gold-ground or gold-size:—

Let twenty-five parts of linseed oil be boiled for three hours with one part of

[1] The author has renounced the practice of putting up preparations in this fluid, and permitting the access of air, for reasons given at p. 2, line 29, et seq.—[Trans.]

red lead (*menie*) and one-third of a part of umber, and then poured off. Next take white lead and yellow ochre, well pounded and divided, and mix them together in equal proportions. Successive portions of this mixture must be added to the oil, and well rubbed up and mixed with it, till a tolerably thick fluid is formed, which must be once more thoroughly boiled.

If now a preparation has been made, which it is wished to preserve in the chloride of calcium, or any of the five last-mentioned fluids, and if it can, without injury, bear a little pressure, the following manipulation is recommended:—

If the specimen is moistened with water, which during the prelimnary examination is frequently the case, all superfluous fluid is in the first place removed with a little roll of bibulous paper, or with a camel-hair pencil, such as I have elsewhere recommended. The fluid at a little distance from the object may be wiped off with a cotton or linen rag, and the surface of the glass there made perfectly dry. A certain quantity of the preservative fluid is then placed upon the specimen, and this is most conveniently effected by using a dropping-flask. The amount of fluid should be such that it should afterwards perfectly fill the space beneath the covering plate; the proper quantity is soon learned by a few trials. Next a (square?) covering-plate, about two millimetres ($\frac{1}{12}$th of an inch) narrower than the object-slide, should be laid under the centre of the latter,—*i.e.*, immediately beneath the part which it is destined to cover. A pencil is next dipped in the cement, and a square drawn with it upon the glass around the fluid containing the specimen, so that the cement shall extend from one to two millimetres ($\frac{1}{25}$th to $\frac{1}{12}$th of an inch) within the margins of the covering-plate. The latter is now to be placed upon the specimen, and its margins finally covered with the cement. If there is too much fluid beneath, the superfluity finds a channel for escape; an opening then takes place in the cement, below the cover, but is again closed, if care be taken to renew the application of the cement to the edges of the cover, when the superfluous fluid has been removed, or has dried up. In about two days, the outer layer of the luting will have become dry, but the inner layer remains soft for many weeks and even months. This is just what constitutes the excellence of the cement, for it never bursts and permits evaporation; and a great number of preparations which I have put up in this manner are at the present time, after the lapse of several years, quite unaltered. It is, however, of importance that the cement shall occupy a portion of the space between the object-plate and its cover; a mere anointing of the edges of the latter is never sufficient.

If the specimen be one which will not bear pressure without injury, it must be put up in some kind of cell, the depth of which must be regulated by the thickness of the object. The covering-plate must in this case be always smaller in diameter than the space between the outer margins of the cell. First, some preservative solution is placed in the cell, and then the object is laid in it; the upper edges of the cell are then touched with a little of the *gutta-percha luting*.[1]

[1] The reader will find the receipt for this composition, and directions for making cells of gutta-percha and caoutchouc, at the end of the present article.—[Trans.]

The cell is then completely filled till the fluid even forms a convexity above its margins; if now the cover is applied, the superfluous moisture escapes, and no air remains in the cell. Finally, when the edges are dry, they must be covered with a thick layer of the luting, and with a second a few days afterwards.

The method last described is especially applicable to the preservation of injected specimens in a solution of arsenious acid.—Vol. ii. p. 350-355.

Preparation of Caoutchouc Cells.

In commerce we now obtain caoutchouc plates of different thicknesses. The thinnest measure about one millimetre ($\frac{1}{25}$th of an inch), and out of these plates of any required thickness may be formed, as their surfaces adhere perfectly together, especially if previously slightly heated. In a square piece of suitable thickness an opening may be made by means of a scissors, or the centre may be cut out of a disc-shaped piece by means of a hammer and ring-shaped punch. To fasten the caoutchouc ring to the object-slide we use the following luting:—

One part of finely-cut gutta-percha is mixed with fifteen parts of oil of turpentine, and dissolved in it by gently heating, and constantly stirring, the mixture. The solution is then poured through a cloth, to separate some impurities which are always to be met with in raw gutta-percha. To the purified solution there is added one part of shell lac, which, by the aid of gentle heat and constant stirring, must be dissolved in it. The heat is then kept up until a drop of the solution let fall upon a cold surface becomes nearly hard. The cement is then ready for use. If it is afterwards found requisite to melt it again, a little oil of turpentine should be added before applying the heat.

To attach the caoutchouc ring to the glass, proceed as follows:—Lay the ring upon the table, and above it place the glass object-slide, so that the ring occupies the centre of the slide, and a free margin of glass is left around it. A pencil is now to be dipped in the warm luting, and carried over the portion of the glass through which the ring is seen, care being taken to spread the luting in a thin layer, as the superfluous fluid would otherwise flow out from the edges. The ring is now removed from beneath the slide, and laid upon the spot marked out for it with the cement. The plate is next warmed by holding it over fire, and then laid, ring downwards, on a cold piece of mirror glass till the cement has become cool and hard.

Gutta-Percha Cells.

Gutta-percha, which, like caoutchouc, resists the action of almost all chemical agents, has, besides, the property of becoming soft and plastic in warm water, and can thus be fashioned into any required shape, which it retains on cooling and resuming its former consistence. Gutta-percha sheeting may be procured in commerce, like caoutchouc sheeting, of any thickness, and will be found very useful for microscopical purposes. Plates of this substance may be provided of various thicknesses, according to the required depth of the cells,—for example, from $\frac{1}{10}$th of a millimetre to three millimetres ($\frac{1}{250}$th to $\frac{1}{8}$th of an inch) in thickness. These plates must then be cut into square pieces, a little narrower than

the glass slides on which they are to be fastened. The openings may be cut out with a scissors, or struck out with a punch and hammer, the plate being laid upon a piece of cork. To fasten gutta-percha rings to the glass plates the cement recommended for caoutchouc is employed, and the process conducted in the same way, with this difference, that, after the last heating, which makes the gutta-percha soft again, pressure should be made upon it for a few seconds with a cold piece of mirror glass. The upper surface of the cell is thus rendered quite flat and smooth, so that the glass cover, when applied, is everywhere in contact with it. In this respect the gutta-percha cells are preferable to those of caoutchouc, the upper surface of which, especially about the edges, has always some degree of convexity.—Vol. ii., pp. 125-127.