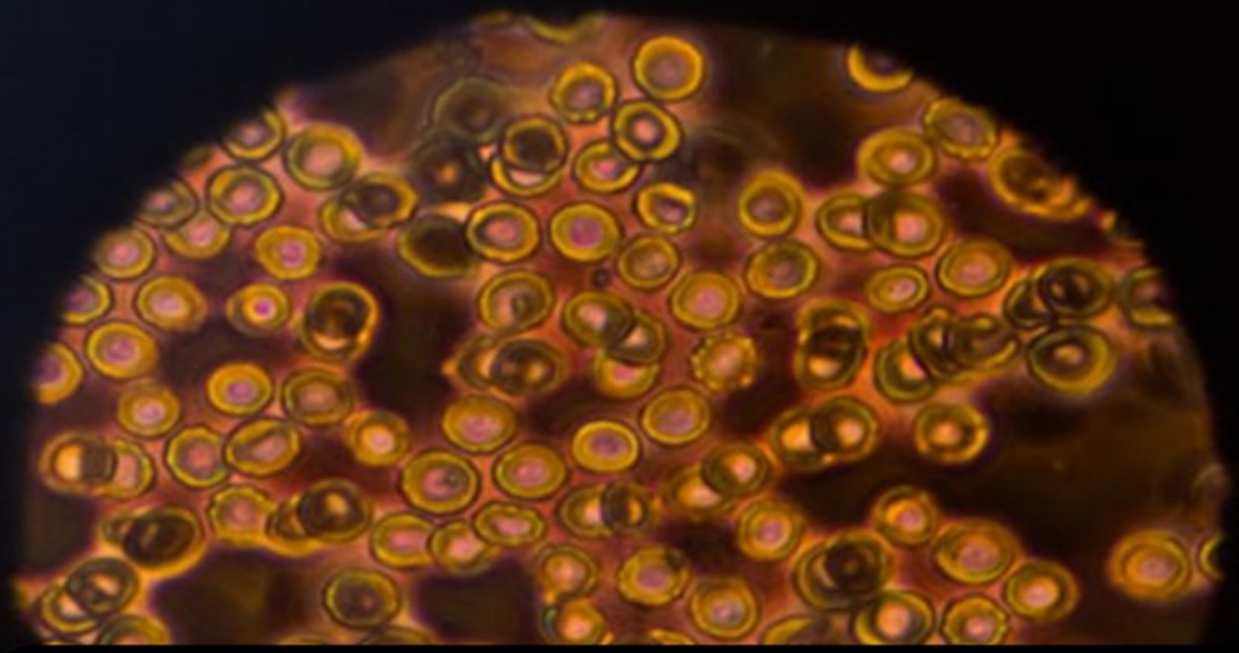
WALTER S. COLMAN



SECTION CUTTING AND STAINING

Walter S. Colman

Section Cutting and Staining

A practical introduction to histological methods for students and practitioners

EAN 8596547220930

DigiCat, 2022
Contact: DigiCat@okpublishing.info

Table of Contents

CHAPTER I.

Table of Contents

Apparatus Required.

Table of Contents

Probably there is nothing more perplexing to a beginner than to decide what apparatus is required. If he consult a price list, it is difficult for him to tell which articles will be necessary, and which will be either luxuries, or required only for special investigation.

In the following account of requisites, those only will be described which it is useful to have always at hand. They will be found sufficient for ordinary work, but for special investigations a more elaborate equipment will be required.

All staining and other reagents should be made as far as possible by the worker himself, according to the directions given in later chapters. This should at any rate be done at first, as the knowledge thus gained will prove invaluable. It will also effect a great saving if articles that are used in any quantity, such as methylated spirit, distilled water, &c., are bought by the gallon, and not in small quantities.

Almost all the processes described here can be carried out without the use of a fully equipped laboratory, in fact, in an ordinary room. The only furniture required is a firm table, and a cupboard and shelves for storing reagents.

The following should also be procured:—

Jars or **bottles**, with well fitting stoppers or corks, to contain the tissues while being hardened. They should not hold less than two ounces. Empty drug bottles which can

usually be obtained from druggists for a few pence, serve very well.

Smaller bottles should also be procured for keeping specimens in spirit after they have been hardened until one is ready to cut sections. After sections have been cut from a portion of the specimen, the rest should be preserved, in case it is wanted for further investigation. Each specimen must be labelled, with a name or a number corresponding to a reference in the note-book, and a large number of specimens may then be kept in the same jar. The best way to label them is to write the name or number on a piece of vegetable parchment in ordinary “marking ink,” and warm it until the writing is black. The little label should then be fixed to a corner of the piece of tissue with a stitch or by a fine pin, and it may be identified years afterwards. The importance of keeping tissues, sections, slides, &c., **distinctly labelled** cannot be too strongly impressed on the beginner. The name, date, and other particulars should be invariably written on the label at the time. At first the student will be inclined to neglect this, as he will recognize his pieces of tissue and sections so readily merely by their shape and general appearance. But as time elapses and similar specimens accumulate, he will find it most difficult or even impossible to identify one from the other.

A number of 1oz. and 2oz. **stoppered bottles** for staining reagents.

The stopper of these should be fitted with a rod. This is done by simply heating the lower end of the stopper and the upper end of a piece of glass rod of suitable length in a

blow-pipe, until they are plastic, and then pressing them together.

Watch glasses.—At least a dozen watch-glasses, in which to perform the operations of staining, clarifying, &c. Those with a flat bottom should be employed as they are less easily upset than the others.

Plenty of **filter papers**.

Both coarse ones, for use in the manufacture of reagents, and small fine white ones (21/2inch) for filtering the staining fluids immediately before using them, should be procured. Before using them a few drops of alcohol or distilled water should be placed in them to saturate the paper. This not only allows the fluid to pass through more rapidly, but prevents a portion of it being wasted through being absorbed by the pores of the paper.

Several **needles** mounted in handles.

They must be kept very bright and smooth, and care must be taken that the point does not get turned up.

A large and small **funnel**.

Several **pipettes** consisting of pieces of glass tube with an internal diameter of 1/8″ and about ten inches long, drawn out almost to a point at one end.

Section lifter.—This instrument is required for transferring sections from one reagent to another, or from oil of cloves, &c., to the slide. The most convenient form is Woodhead’s, made of thin sheet copper, which allows the blade to be bent at any angle to the stem. The stem or handle is about six inches long, and continuous with, and at an angle to it, a flat blade about 3/4in. square with the corners rounded off. Larger ones can be obtained for

mounting sections of large size, *e.g.*, kidney, medulla oblongata, &c. The surface of the blade should be brightly polished, and kept scrupulously clean.

Ordinary dissecting **forceps**.

One or two **scalpels**.

A pair of fine **scissors**.

A **razor** or other instrument for cutting sections.

A smooth **oil stone** for keeping the razors and knives properly sharpened.

A **spirit lamp** for warming the staining fluids.

A few **test tubes**.

A **minim measure**.

Scales and small weights.

A gross of ground glass slides 3 x 1in.

Half a gross of ground glass slides 3 x 11/2in.

Half an ounce of thinnest coverslips, 7/8in. diameter.

Quarter of an ounce of thinnest coverslips, 11/4in. diameter.

Microscope.—This is not the place for a description of the microscope as an optical instrument, but some hints as to the selection of one may be found useful.

Showy microscopes with much brass work should be avoided, simplicity of construction being a great recommendation. The microscope should have a large heavy base, either of the horse-shoe or tripod pattern, large enough to afford a firm base when the microscope is tilted.

Mechanical stages are unnecessary and they add greatly to the expense, and very little to the utility of the instrument for ordinary histological work. Binocular arrangements also are of little use for this purpose.

The microscope should be provided with a **coarse and fine adjustment**, which should be most carefully tested before purchasing the instrument. They should work freely and smoothly, and the slightest turn in either direction should at once alter the focus.

There should be a **reversible mirror**, one side being concave and the other plane. The concave surface is the one usually employed, the plane surface being chiefly used in conjunction with the sub-stage condenser for the examination of micro-organisms. There should be an **eye-piece** of moderate magnifying power. Very powerful eye-pieces do not reveal additional details, but merely enlarge the image, and with it any defects that may be produced there by faults in the objective. Eye-pieces II. and IV. of most makers will be ample for most requirements.

Objectives.—These are the most important parts of the microscope, and the student will be well advised if he spends a little extra money to secure good lenses.

Most objectives and stands are now made with a universal thread, so that any objective will fit any make of stand. Many workers provide themselves with a cheap stand such as that supplied by Leitz, and then fit it with lenses by Zeiss, or other first class maker.

The most useful lenses are the 1in. low power lens, and 1/5in. or 1/6in. high power, or No. 3 and No. 7 of Continental makers, or Zeiss's A and D. A 1/2in. lens will also be found very useful.

For minute work, such as bacteriology and blood investigations, higher powers will be required, 1/8 or 1/12 **immersion lenses**. These objectives come extremely close

to the object, and very thin cover glasses must be employed. In order to avoid the refraction caused by the rays traversing the air between the coverslip and lens some immersion fluid is placed between the two. With some lenses water is employed, but usually an oil having the same refractive index as glass is used, and the one most generally employed is cedar oil (Zeiss prefers the oil from the species Juniperus virginiana). A spot of oil is placed with a rod just over the object to be examined and the objective carefully lowered by the coarse adjustment till it comes in contact with the droplet of oil. The focussing should then be managed with the fine adjustment only.

When the section has been examined the oil must be removed from the lens. For this purpose a soft silk handkerchief or a special piece of chamois leather may be employed, and used very gently. If all the oil cannot be removed, the handkerchief may be moistened with a little absolute alcohol, and the lens hastily wiped. The alcohol must not be allowed to remain in contact with the lens as it is a solvent of Canada balsam with which the lenses are often cemented in position.

FIG.1.—Double or Triple Nose-piece.

Double or triple nose-piece (fig.1).—This mechanical arrangement is placed on the lower end of the tube. Two or three objectives of different magnifying power are attached to it. The nose-piece rotates round a central pivot in such a way that the objectives can successively be brought accurately into position above the object on the stage. It is, therefore, a moment's work to replace a high power objective by a low power one and *vice versa*. It is an extremely convenient time-saving appliance, and by its use the risk of dropping and injuring the objectives when screwing them on and off frequently is avoided. Those whose microscopes are not already fitted with this appliance can easily have one fitted on at a cost of about a sovereign.

Substage condenser.—This mechanism for concentrating light on the object is a necessity for bacteriological work. The most convenient form is **Abbe's illuminating apparatus** (fig.2).

This consists of a system of short focus lenses which collects the light received by the mirror, and throws it on the object. The amount of light received from the mirror is controlled by an "iris diaphragm," the aperture of which can be dilated or contracted by moving a small lever at the side. It can be fitted on to most microscope stands, but it is better to get a stand in the first instance which is constructed to carry one.

The cost of a microscope varies from two guineas to two hundred. There are many excellent microscopes in the market, and of these several may be mentioned which the writer has found to work satisfactorily.

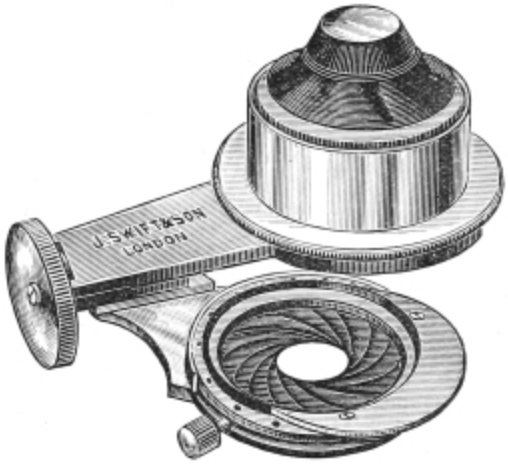


FIG.2.—Abbe's Illuminating Apparatus.

Of the cheaper student's microscopes the "Star" microscope made by Messrs. R. and J. Beck, of Cornhill, E.C., will be found a safe investment. It may be obtained with coarse and fine adjustment, nose-piece, and 1in. and 1/4in. objectives, for about £5. Those who require a better instrument will find Beck's "Pathological" microscope fitted with nose-piece, Abbe's illuminator, &c., for £16, meet all requirements.

Leitz of Jena, supplies two good and cheap microscopes for £310*s.* and £5. They are not, however, of uniform excellence, and they should be carefully tested by some competent judge before the purchase is completed. Leitz immersion lenses are cheap, and often extremely good, but should be carefully tested beforehand, as their quality is not quite uniform. The microscopes can be obtained from Mr. A. Frazer, Teviot Place, Edinburgh.

The "Bacteriological" microscope, made by Messrs. Swift, of Tottenham Court Road, is one with which no one can be disappointed. It is sold with Abbe's condenser, triple nose-piece, 1/6in., and a 1/12in. immersion objective, for just under £20. Both stand and lenses are turned out in Swift's