



## NOTES ON MICROTECHNIQUE

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### 1. HANDLING NUMEROUS SMALL OBJECTS FOR TOTO STAINING, EMBEDDING OR STORAGE

In working on a problem which involved the repeated handling of numerous fish embryos of the same and different stages of development, the time consumed in keeping specimens separated in vials, while dehydrating, etc., seemed prohibitive and so efforts were made to devise a better method.

The following procedure served the purpose very nicely: Short pieces of glass tubing, 5-7mm. in diameter and about 1 inch in length, were plugged loosely with absorbent cotton. A piece of card was cut the same width as the tube and labeled with the number used to designate the embryonic stage and sample. This card, together with the specimens, was then placed within the tube in the particular fluid to be used (water; alcohol or fixative). Many such tubes were placed on end in a small jar

and covered with the fluid. Changes in the medium, such as occur in dehydration, were easily accomplished by pouring off the fluid of any one stage and adding that of the next. Fewer steps in the series were possible since the cotton retarded the diffusion currents and some of the material from the previous step remained in the tubes.

Not only dehydration but also infiltration with paraffin, was accomplished in the same fashion. In our laboratory the embryos are kept in the tubes throughout fixation, etc. until ready for imbedding. More than 1,000 specimens of 200 odd stages and fixations have been stored in the same pint jar. The number and size of the objects determines the diameter and length of the tubes. They should not be much over 7 mm. in diameter or 2 inches in length for the most efficient results. There seems to be no advantage in having extra space in the tubes. The cotton plugs should not protrude from the tube and should be fairly loose, i. e. just tight enough to prevent the specimens from falling out when the tubes are handled with forceps during transfer. Usually the fluids are poured off and others poured on without removing the tubes from the container.

In paraffin infiltration only 15-20 tubes can be handled at one time efficiently since the specimens in each tube must be embedded separately.

A medicine dropper was found satisfactory for transferring objects to the tubes after fixation.

## 2. LABELS FOR PARAFFIN BLOCKS

After getting the embryos ready for imbedding, the problem of marking the paraffin block so it can be quickly identified arises. This has been most satisfactorily solved by using India ink on the label card in some numerical system. This ink, when allowed to dry before immersing it in fluid, does not fade in the majority of reagents used. In imbedding, the label card is placed face down in the dish and the paraffin is poured over it. When the block is removed from the dish or holder the label is thus in plain sight. In preparing a block for sectioning, the number can be shaved off.

## 3. ELIMINATION OF AIR BUBBLES IN PARAFFIN IMBEDDING

Trouble in imbedding material because of air bubbles is quite common. This is sometimes very perplexing, but care in the following procedure will tend to alleviate the difficulty.

(a) Warm the imbedding dish until it is at the same temperature as the paraffin before pouring melted paraffin into it. The paraffin must be kept melted until imbedding occurs. This is important.

(b) Always change to a clean, hot dish, and fresh, melted paraffin, when imbedding material. Paraffin in melting always seems to form bubbles and when the imbedding dish is cool the paraffin solidifies, around the outside at least, and thus must be remelted before imbedding can occur. Overheating of the paraffin and failure to completely displace the Xylol are also common causes of bubbles in imbedding.

## 4. THE USE OF AN ELECTRIC SOLDERING IRON IN IMBEDDING

Orienting specimens while cooling the paraffin is sometimes a tedious and time consuming task. The author has found that an electric soldering iron is of great assistance in doing this more efficiently.

The soldering iron is clamped in a holder on a ring stand so that the imbedding dish can be easily slipped under it and the surface of the paraffin will be a millimeter or so below one face of the iron. Ice water placed around the imbedding dish congeals the paraffin on the bottom

and sides while the heat from the iron retards a too rapid cooling of the surface. Needles in contact with the iron carry the heat into the paraffin and enable one to manipulate the object even though the paraffin has begun to solidify.

A small electric iron costing 50 cents at one of the dime stores is being used quite satisfactorily.

##### 5. MICRO-PHOTOGRAPHS WITH A STRIP-FILM CAMERA

The idea of using a strip-film camera originated from a note on "Photomicrographs with a Vest Pocket Kodak" by F. M. Turrel in *Science* 1933 p. 267.

Following the suggestions given in the article the author, together with James Bragg, devised some simple holders for the "Memo" camera. The basic principle is the use of a wooden block so attached to the eyepiece that the barrel of the microscope carries the weight of the camera. A wooden frame holds the camera so that it is exactly centered above the eyepiece. Since the memo is almost perfectly flat on the face, it is a comparatively easy matter to focus the microscope and then place the camera in position and make the exposure. Using photo flood lamps the usual exposure is from 5-15 seconds and thus it is practical to photograph only stationary materials.

Focusing can be accomplished in several ways: (a) by the use of the demonstration eyepiece, (b) by using a binocular microscope and focusing through one eyepiece, (c) by focusing directly and tightening the microscope ratchet so that the slight jar of placing the camera in the holder will not affect the focus.

The advantage of the strip film camera seems to the author to be two fold. (a) The cost of film, etc. is much less and, (b) because the image can be greatly enlarged, lower magnifications can be used in the microscope and thus greater clarity and depth can be obtained. The author has found the wide field binocular most useful in this respect.

Holders were devised by Mr. Bragg for a Leitz wide field binocular and a B. & L. demonstration eyepiece.

