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a new method of enclosing and preserving small objects for microscopic examination

Adolpho Lutz
A new method of enclosing and preserving small objects for microscopic examination*

The method of including objects for microscopic examination between slide and cover-glass is so generally used that no one would think of substituting it by another process.

In fact it is certainly the best process when it is a question of microscopic sections or flat bodies. When, however, the objects are thick, concave slides or cells have to be used and there arise many inconveniences, specially for use of liquid media as it is shown in the case of many small animals, which either dead or alive take a certain position depending on their shape; sometimes it is, however, necessary to examine them in other positions and that is only possible under great difficulties. In any case, in a shut preparation, the body can only remain in one position.

I soon realized this drawback. It was when I started my very first research work, forty two years ago. It resulted in a prize-winner essay on Cladocera found in the neighbourhood of Berne. To study the morphologic characters of this group, specimens have to be examined in different positions, whereas if left to themselves they generally take a position which only shows their lateral surface, which becomes very inconvenient.

So as to avoid this nuisance, I enclosed my specimens in capillary tubes in which they could be turned around in any direction; I then found that the capillary tubes, when placed on a slide, could only be examined with a low magnifying power, as the rays of light are broken when they pass through different refringent media and are refracted irregularly, so that a correct image is not seen.

The defects of the class and the reflection of incident light disturb the distinctness of the image. To remove these obstacles it was only natural that I should think of immersing the capillaries in liquid. In this way the refraction of the rays was so much diminished that the highest power could be used. The liquid, contained in a small glass dish with flat bottom, has uniform thickness; the only deviation of the rays is caused by the difference of refraction of the media through which they pass and which in these cases consist of:

1° – Bottom of the glass dish;
2° – Liquid in which the capillary is immersed;

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* As a pamphlet or insert, this paper came out together with “On the use of phenol (carbolic acid) in microscopic technic” (p.115-6). They have been separated in the present republication.
3° – Lower wall of capillary;
4° – Liquid enclosed in capillary;
5° – Upper wall of capillary;
6° – Immersion liquid which covers the capillary;
7° – Stratum of air existing between the surface of the liquid and the focal lens of the objective.

When the light which traverses these layers is reflected by flat mirror, it assumes a direction, almost perpendicular to the media passed though and this excludes a marked deviation. The difference of refraction in the glass however and in the different liquid media is not very great, being much less than what takes place between these and the air. By this process, the irregularities of the glass and the reflection of the capillary are reduced to a minimum, so that clear images of the objects can be obtained, that is when they are not in direct contact with that lateral walls of the capillary. (Fig. 1 and 2).

To demonstrate this difference, an empty glass tube may be put in an empty glass dish; it is clearly seen. This also happens when the capillary full of air is submersed in a layer of liquid. The capillary full of water is hardly seen at the bottom of the dish covered by a layer of water.

The liquid of capillary and the liquid in which it is submersed have the same index of refraction, the capillary is almost invisible like a piece of ice without air-
bubbles, immersed in a glass of water. If, for example, the liquid is cedar-oil or any other immersion-oil, the wall of a thin and completely transparent glass capillary will have not any effect on the luminous rays, and allows the use of an immersion-lens. When the focal distance does not allow very high power, still objectives and eye-pieces which magnify hundreds of times may be used allowing the observation of the most important details in the determination of species.

When the indices of refraction of the internal and external liquids of the capillary are identical, it does not matter if they are slightly different to the index of refraction of the glass that forms the wall of the capillary, as experiments will easily show. In studying zoological and botanical specimens, there may be a difference between the external and internal liquids without diminishing the distinctness of the image while using the usual magnifying powers. Under these conditions, it is possible to use any kind of liquid inside the capillary, as for instance, fresh or salt water and physiological solution for living organisms; and for the dead ones, any fixing media as alcohol, formaldehide solution, glycerin and finally clearing media, such as phenol mentioned in the proceeding article, cedar-oil, etc. In the first case the outside liquid in the glass dish must be water, which may also be used with alcohol and solutions of formaldehide. As medium of higher refraction I use glycerin, which is not good for practical uses; in some cases it may be substituted by common or essential oils, liquid paraфин, etc.

Capillaries of transparent glass are easily found as they are used for vaccination purposes; they may be made by drawing heated glass tubes the same way as pipettes are made. The most suitable thing is to have perfect glass, and perfectly cylindrical tubes.

The glass is not necessarily of a given thickness, it must however be uniform in each capillary and sufficiently resistant. The cylindrical shape guarantees the easy examination of the objects, as the capillary may be rolled on the bottom of the glass dish. In some cases, however, more flattened shapes, rectangular or oval, offer greater advantages. The caliber of the tube ought not to be much thicker than the object enclosed in it. I generally use from 1 to 5 millimeters, but in some cases it may be twice that size. For larger dimensions it is advisable to reduce the thickness of the objects through longitudinal sections. If the tube allows it, a parchment label may be placed inside.

To get the object inside the capillary, capillarity may be used or it may be sucked in by the mouth or by any other means of suction. The object may be adjusted by means of bristles or mandrins for the needles of syringes or very fine capillaries. They may be sealed with paraфин or better still shut by heat of flame; in the latter case the ends must be rounded and finer than the body of capillary. To facilitate the enclosing, the ends may first be drawn out, leaving a tiny hole. It will be quite easy to close it after having put the object inside.

A capillary or a pipette may be used to fill the capillary. A very easy and simple way however is to put the capillary in a centrifugation tube full of the liquid, which is to be used. One turn of the centrifuge drives out the air and lets in the liquid. Before closing the tube, the excess of liquid must be driven out by heat or by means of a fine capillary. Below the fusion-point, an air space of 0.5 cm. must be left and of larger dimensions in the case of inflammable liquids. As to the
length of capillary, it may be more or less the length of a slide, so that it may be inserted in pieces or cardboard cut according to the size of the slides generally used in microscopy. The cardboard must be flexible, about the thickness of visiting-cards. The capillaries are held in place by incisions as indicated in figures 3 and 4, the writing can be directly on the cardboard, thus avoiding the necessity of a label. The tubes can then be put away with the preparation of slides and cover-glasses. The capillaries may also be kept in glass-tubes, like the glass tubes in which insects for collections are kept. When neatly closed by fusion, no drying-up needs to be feared.

If the object thus enclosed is not in the right position, either nearer to one or the other end of the capillary, centrifugal force will dislocate it as desired.

For the objects that one wants to keep, a preserving liquid of equal parts of water, glycerine and alcohol with a certain percentage of phenic acid, pure or diluted glycerine or pure phenol may be used. When the objects are opaque the latter is far superior to any other; if it turns red it may be replaced by guayacol, without changing the tube. A little opening at one end and the centrifuge will quickly replace phenol by air and air by guayacol.

I cannot here, for lack of space, mention all little artifices used by me in employing this method. I will merely say that in some cases it may be advisable to move the capillary in the liquid with the finger-tips so to be able to examine it, and in other cases to fix the capillary at the bottom of the glass dish. The objects enclosed in capillaries that are not very wide, do not get out of place by rotation. If however, you

Fig. 3 – Larger tubes, with larvae of Blepharoceridae, in phenic acid, fixed on cardboard. When immersed in glycerine, magnifying powers may be used so as to show the internal structure clearly.

Fig. 4 – Same.
want to immobilize the tube, it may be fixed at the bottom of the glass dish by
paraffin or by imbedding its end in a piece of leaden tubing full of paraffin.

Another medium for fixing the object is glycerine-gelatine; the objects may
thus be examined in liquid through a little heating of the tube, and then they can
be fixed in the desired position by letting the tube cool.

I may yet say a few words on the originality of this method. There is nothing
here absolutely unknown, but I have never read or heard of an exactly identical
method. It is, as here exposed, perfectly suitable for the ends for which it is meant.
I am sure however that this method might be still improved, if it were worked out
jointly by myself and a maker of optic instruments and glass objects.
Same (larva of the species *Curupira mochlura*), retouched so as to remove the outline of the walls of the capillary tube. BR. MN. Fundo Adolpho Lutz, caixa 36, pasta 247.
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