CHAPTER 14a

The Taylor Permanent Microscope Slide

BACKGROUND

An entire volume could be written about all the slide mounts that have been designed since the beginning of microscopy. Fluid mounts of specimen in aqueous media have been especially problematical.

One of the methods, which held great promise, came into use in the early 1930s. Murrayite was used as a sealant. Unfortunately, many slides sealed with it have deteriorated due to its incompatibility with glycerol. The sealant gradually encroaches the area of the medium as it absorbes the glycerol. It is not uncommon to find that this process has resulted in the sealant infiltrating the specimen, making it difficult to view. Aqueous slide mounts in some important collections are deteriorating at an alarming rate and there is a great need for improvement.

OBJECTIVE

My new procedure was refined over a number of years with attention given to chemistry, mechanics, optics and ease of execution. Study of my own collection of slides sealed and ringed with Murrayite, and reports about the condition of such slides in other collections led me to set the following objectives:

1.- The microscope slide should be a standard, 75 X 25 mm with a thickness under 1.2 mm.
2.- The microscope slide should be glass, plain, without cavity, precleaned and fire polished .
3.- The cover glass should be round, of #1 quality under 0.17 mm thick.
4.- The sealant should not require solvent volatilization for setting.
5.- The sealant should be compatible with water, formal, phenol and glycerol.
6.- The cover glass should be supported only at three points by such means that the underside is always close to the specimen for best resolution.
7.- The procedure should preclude glass surface contamination that could inhibit adhesion of the sealant.
8.- The ringing compound should be compatible with the sealant and not become brittle in time.

OVERVIEW

While this method was developed to meet the needs of preparing permanent slide mounts of rotifer whole forms and their trophi, it is equally applicable for other microinvertebrates.

The fourth and fifth objectives were met by Norland Optical Adhesive NOA 61 (1) as the prime sealant and final support. It is a clear, colorless, liquid photopolymer that is cured by ultraviolet light. The cure is not inhibited by oxygen, hence areas in contact with air at the periphery of the preparation will cure to a non-tacky state. My procedure replaces older ones which used supporting rings of cement or aluminum.

The sixth objective is met by a two-stage support system in which three short lengths of pyrex filament, are used to support the cover glass during precure of the sealant, after which they are withdrawn. At this point, NOA 61 takes over the support function and the curing continues.

The seventh objective is met by the behavior of NOA 61 in contact with the aqueous mounting medium cell. It flows around the cell by capillary action, even replacing areas where the medium may have been.

The eighth objective is met by using two ringing compounds by Northern Biological Supplies Ltd (2). The cured slide is ringed with the completely waterproof ringing compound Clearseal One followed by the protective ringing compound Bioseal Mountant Two.

GENERAL DESCRIPTION OF THE PROCEDURE

A slide is placed on a ringing table having an underlay scribed with a circle of the exact diameter of the cover glass to be used. Three short lengths of pyrex filament of selected diameter are cemented to the slide, equally spaced and with one end extending 2 mm into the scribed circle.

A metered quantity of mounting medium is placed at the center and the specimen is transferred to it.

The cover glass is lowered onto the mounting medium and on down to rest on the supports. The amount of mounting medium is such that it spreads to within 3 mm of the cover glass edge and within 1 mm of the inner end of the supports.

The cover glass is affixed to each of the supports in order to prevent it from being raised when NOA 61 is flowed under it.

The NOA 61 is run under the cover glass, up to and all around the mounting medium, filling the entire 3 mm space around the mounting medium out to the edge of the cover glass.

The preparation is set aside, exposed uniformly to ultra violet for a brief time until precure of the NOA 61 has occurred, setting the bond sufficiently so that there is no danger of disturbing the alignment of the cover glass.

When precure of the NOA 61 has been obtained, the three filament supports are freed and withdrawn, thus leaving the support to the partially cured NOA 61.

The preparation is returned to ultraviolet exposure for a longer cure to obtain full crosslinking and solvent resistance of the NOA 61.

The slide is ringed with a waterproof compound followed by ringing with a protective compound.

INSTRUMENTS

Dissecting Microscope, darkground preferred.
Ringing table — my preference is for a low profile so that it can be used on the stage of the dissecting microscope.
Turntable fixture for wicking. (See below)
Forceps, slender, smooth pointed tips — my preference for being able to pick up very small diameter pyrex supports.
Forceps, serrated tips — my preference for selecting cover glasses.
Forceps, flat, broadened , bent tips — my preference for seating supports.
Velvet, dark — for placing supports and cover glasses in preparation.
Rod-type droppers — for transferring glycerol and cover glass.
Diaphragm pipette — my preference for metered transfer of water.

MATERIALS

Microscope slide, 75 X 25 mm.
Cover glass, 12 mm diameter, #1.
Glass rod, pyrex, 3 mm diameter.
Rubber Cement
Epoxy, 5 minute, two-part. (See "Choosing Epoxy" page 8)
Toothpicks, round.
Norland 61 Optical Adhesive, Norland Products, Inc. (1)
Clearseal One, Northern Biological Supplies. (2)
Bioseal Two, Northern Biological Supplies. (2)

PREPARING THE INSTRUMENTS

Rod-type droppers with spherical ends of various diameter are needed. They provide the only way to transfer glycerol for which a pipette should never be used. I draw them from 3 mm diameter pyrex rod. I make them in two steps. First, I draw one end of a 10 cm length down to 1 mm over a length of 1 cm. Second, I form a spherical tip as shown in Figure 4. It is important that the transition from the 1 mm diameter to the sphere be sharp in order to deliver reasonably consistent quantities.

Several such droppers need to be made because the quantity of glycerol to be transferred is determined by the volume of the medium under the cover glass. That is determined by the diameters of the cover glass and the supports.

Pyrex filament supports of various diameters are prepared. I draw 20 cm lengths from 3 mm diameter pyrex rod down to diameters ranging from 0.05 to 0.5 mm and store them in a transparent plastic tube for selection by diameter later and nipping down to 7 mm lengths.

A Ringing table is essential. It should be of good quality and turn freely. It should provide accurate location of the microscope slide with repeatability. For this, my preference is for the three-pin method, i.e., two pins to locate the long side and one to locate the short side. The slide is located in place against the pins, clamped with clips and marked so that the same sides are always brought in contact with the pins.

I have designed for myself a low profile table which can be used on the stage of my dissecting microscope for ringing and for remounting slides. A detailed description of this table is given in Chapter 19b.

Because perfect centrality of the preparation is not required when running the NOA 61 under the cover glass, I have designed for myself for this procedure a very low profile turn table made of poster board stock. It clamps in place on the stage of the dissecting microscope. Some magnification is very helpful.

Chapter 14b gives a detailed description of this table.

STEP BY STEP DIRECTIONS

The following directions describe the way I prepare slides.They are written in first person as step by step directions.

1.- Place a slide on the ringing table. The slide should be marked so that its orientation will be the same when it is returned to the table for later operations. The slide underlay should be marked with a circle exactly the diameter of the cover glass to be used.

2.- Mix a small amount of 2-part, 5-minute setting epoxy and place three very small drops equally spaced 2 mm outside the underlay circle as shown in Figure 1.

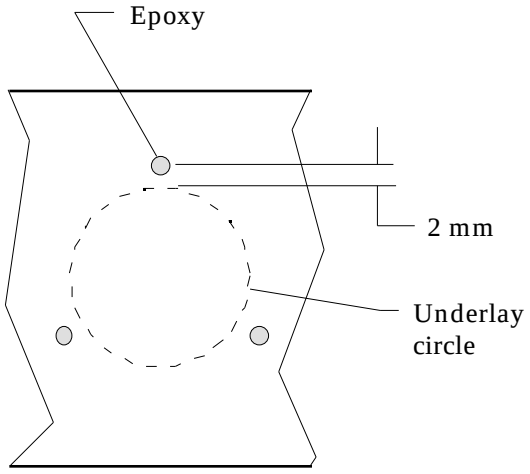

Figure 1. Placement of the epoxy points.

3.- Select a length of filament of desired diameter and nip off 7 mm lengths onto the velvet cloth, which I prefer because it is easy to pick them up.

3.- Select three of the 7 mm lengths of pyrex filament and place one on each of the epoxy points as shown in Figure 2. They will serve as the temporary support and should extend 2 mm into the underlay circle.

Push each support down in contact with the slide using the tip of the flat, broadened, bent tip forceps.

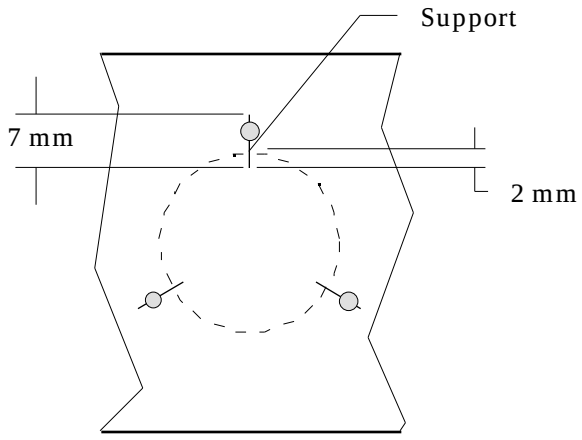

Figure 2. Placement of the temporary supports.

4.- Place a metered amount of the mounting medium as shown shown in Figure 3.

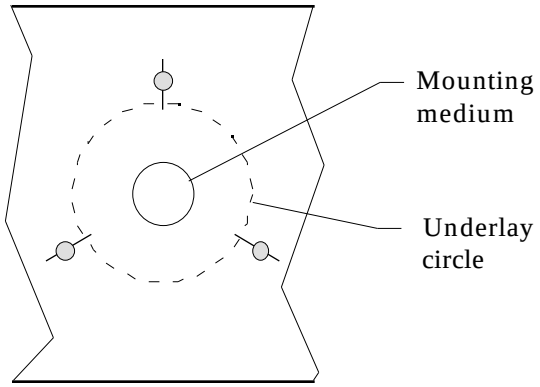

Figure 3. Placement of the mounting medium.

5.- Select a cleaned cover glass and determine which side is convex. It is the side in which the reflection of a distant object is the smallest. Place the cover glass on the velvet cloth with the convex side up

6.- Pick up a very small amount of rubber cement on the tip of a dip rod and give it a moment to dry. Pick up the cover glass by touching it at the center as shown in Figure 4.

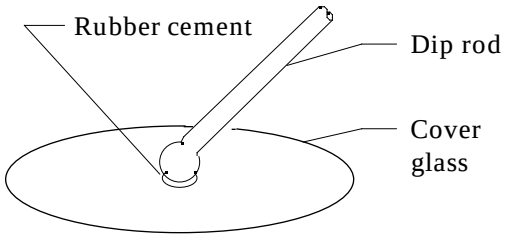

Figure 4. Picking up the cover glass

7.- Bring the cover glass over the supports and the mounting medium. Keep it horizontal and lower it very gently onto the mounting medium and down until it rests on the supports.

Next, hold the cover glass down with a pin in a pin vise and carefully lift the dip rod off without disturbing the cover glass.

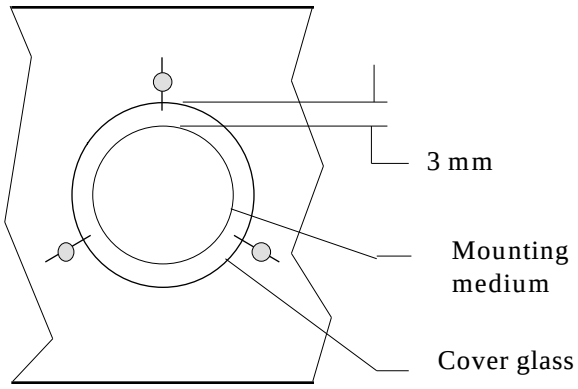

Figure 5. Cover glass in place..

8.- Mix a small amount of 2-part, 5-minute setting epoxy and place three very small drops where the supports pass under the cover glass. The epoxy should be placed on the edge of the cover glass and on the top of the support, but not on the slide.

Set the slide aside for a few minutes for the epoxy to set sufficiently so that the cover glass will not lift up in the following step.

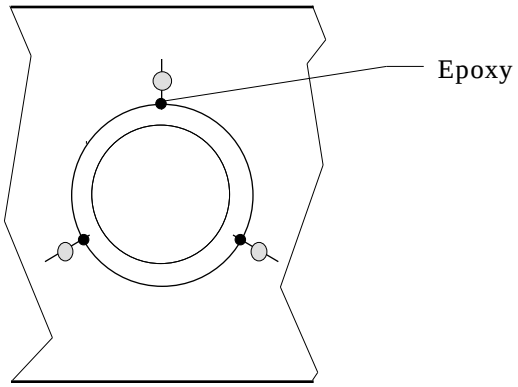

Figure 6. Epoxy points in place

9.- Apply very small quantity of NOA 61 at each of the three supports until just past the inner end, but not in contact with the mounting medium.

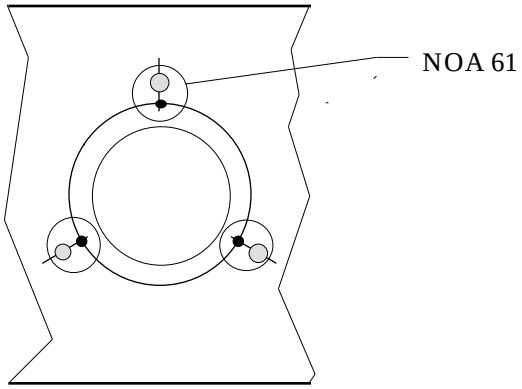

Figure 7. Starting to flow the NOA 61 under the cover glass.

10.- Add enough NOA 61 in equal amounts at each support until the mounting medium is contacted. Repeat this until the entire area outside the mounting medium is filled. Do this quickly and uniformly to avoid displacing the mounting medium cell.

PRECURE AND REMOVAL OF THE SUPPORTS

The slide is now ready for precure by exposure to ultraviolet. The purpose of the precure is to set the NOA 61 bond to the slide and cover glass sufficiently to permit the three temporary supports to be withdrawn without displacing the cover glass.

The removal of the supports is important because NOA 61 has a linear shrinkage of 1.5% during curing. The bond to slide and cover glass would fail if the supports were left in place.

The precure time will depend on the ultraviolet source. In my own work, here in Florida, I place the slide on the window sill of my laboratory window, which faces south east and is free of sun in the afternoon. The precure time takes about one or two minutes. Sufficient rigidity can be observed by slight pressure on the cover glass.

11. Use an X-acto™ blade as shown in Figure 8. Run the tip of the blade along each side of the support to free it from the slide. Do not apply side pressure to the support. Do not remove the epoxy points placed at the edge of the cover glass in step 8.

    Use slender, pointed forceps to grasp each support at the points shown by the two arrows in Figure 8 and draw them out. Avoid lifting or bending them because this can break them and make complete removal very difficult.

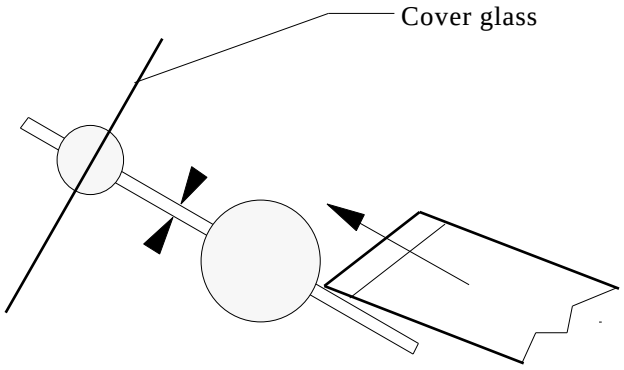

Figure 8. Freeing and removing the supports.

RINGING THE SLIDE

Very low water absorption rate and water vapor permeability make the choice of NOA 61 excellent for the combined functions of primary seal and support. Complete sealing is obtained by ringing with the water proof Clearseal One.

13.- Ring the slide with Bioseal Two which hardens quickly in about one hour and serves to protect the Clearseal One.

The ringing compounds and full instructions for their use are available from Northern Biological Supply Ltd. (2)

CHOICE OF EPOXY

The viscosity of two-part, 5 minute epoxy varies from brand to brand. My preference is for "Elmer's Superfast 2-part by Borden", which I recommend for coverslip replacement of my Microcompressors. Lower viscosity epoxies tend to creep along the supports by capillary action, thus making their removal difficult.

REFERENCES

1.- Norland Products, Inc., 695 Joyce Kilmer Avenue, New Brunswick, New Jersey 08902, U.S.A. See "Modification" below.

2.- Northern Biological Supplies Ltd, 3 Betts Avenue, Martlesham Heath, Ipswich IP5 3HR, UK.

MODIFICATION

The delivery nipple of the Norland applicator is rather large for obtaining a neat mount. Because a minimum of the sealant should be applied outside the cover glass, I insert a short length of tapered tubing made as follows. (1) draw 4 mm tubing down a taper as shown in figure 9. (2) slip on a gage made of aluminum 1 mm thick with a #67 (0.032) drilled hole. (3) place a mark at point **B** and cut at points **C and D** obtaining 2 mm at **C**, 1 mm at **D** and 0.5 mm bore at **D**. (4) finish by breaking the edges at both ends with a fine polishing stone.

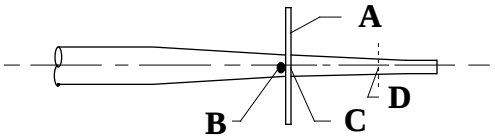

Figure 9. Showing adaptor twice size.

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