Livin' la Vida LOCA

I've been messing around with microscopes since the age of eight but the one aspect of microscopy that has continually frustrated me has been making decent slides, and I'm sure many others involved in this fascinating occupation feel the same way. In the early days the biggest problem I had was in getting hold of suitable materials and chemicals, unless you were in some way professionally involved or had very deep pockets it seemed almost impossible to source fixatives, stains, solvents and mountants. Even getting the basic information presented significant difficulties, at the time I lived in the north east of England which even now can scarcely be said to be a hotbed of amateur microscopists, in my youth I was ploughing a very lonely furrow and essentially gave up the unequal struggle, much to my regret.

Move on a few decades and with the advent of the internet and online buying things have become both simpler and more complicated. Simpler because all the information you could ever want is merely a few clicks away and much of our equipment and consumables can be bought online from anywhere in the world; more complicated because many of the chemicals that were used so casually by the older microscopists are now either not available at all or their sale is highly controlled and quite properly restricted to those who really know what they are doing. In order to get round part of this I've tried using most of the mountants regarded as "safe" and I'm indebted to the Micscape articles written by Walter Dioni in this respect. However, these mountants are often not very durable or limited in application and I've been casting around trying to find something that was easily available and met my personal criteria for a good microscopy mountant, these being;

Safety – the mountant must be safe for use in a domestic environment, as must any solvents used in association with it. Whilst it's not critical, it would be nice if any odours produced by the materials are not too offensive! I'm primarily concerned with any volatiles given off, physical contact can and should be avoided by the wearing of suitable protective clothing and gloves.

Ease of use – I would prefer not to have to heat stuff up to precise temperatures if it can be avoided. Glycerine jelly can be a bit of a pain in that respect, although courtesy of eBay I now have a precision heating block that helps considerably.

Transparency – no mountant is worth bothering with unless it is essentially as transparent as the glass slides and coverslips we use.

Refractive index – the RI should be as close to that of glass as possible, preferably around 1.5. I know that certain subjects such as diatoms benefit from a much higher RI but I'd regard that as a specialist area that personally I don't do.

Speed – I do not want to have to keep slides flat and warm for days or weeks while the mountant sets sufficiently to be able to do anything else with the slide.
Cost – I'm very careful with what I spend on microscopy (ok, I'm mean!) and the cheaper the mountant is the better.
Interaction with stains – I don't want to see stains leaching out from the specimen into the mountant over time, although a small amount of this is acceptable to me.
Shrinkage – ideally a mountant shouldn't shrink at all as this will form voids at the edges of the coverslip but some shrinkage is almost inevitable. The less the better.
Longevity – this is the tricky one because it's only by testing over time that we can judge the performance, although if the mountant has been successfully used in another application for some time this gives us a good indication of whether or not it's likely to work for slides.

Recently I was asked to put together a meetings programme for the Iceni Microscopy Study Group and thought I would volunteer to do a session on safe mountants, using the Walter Dioni articles as a basis. As a club we have made the decision not to use xylene or xylene based materials during club sessions due to potential risks to members by inadvertent exposure, which does rather limit what we can use. While searching for examples of safe mountants I tried to source some Loctite Glass Glue, only to find it does not appear to be in production any longer, although a similar product is available through the high street shop Wilko.

This latter glue meets many of the criteria listed above but it is actually fairly expensive bearing in mind that each tube only holds 3g and costs £2.00 or a staggering £666.67 per KG, which makes it 50% more expensive than sterling silver! Not that sterling silver makes a very good mountant, but it's an interesting comparison, and the glass glue is also more expensive than Canada Balsam, which is generally regarded as a fairly pricey mountant.

The glass glue sets by exposure to UV light and there's also one other material available on the high street that does the same, UV setting nail varnish, and this can be bought for £7 per 15ml bottle, which makes it much cheaper than the glass glue.

I've tried both of these as microscopy mountants and they do meet most of the criteria but I found that both tended to shrink rather more than was acceptable, especially the nail varnish. I could not find an RI specified for either but I suspect it is near to that of glass, especially the glass glue. One interesting aspect of both is that they actually absorb UV light, which has an interesting application I'll mention later. I use either a UV torch that emits at 365nm, or a nail varnish setting device, shown in the photo, that uses a fluorescent tube of unknown specification. Both work well but shining the

torch though a slide made with glass glue or UV nail varnish shows a distinct shadow indicating fairly strong absorption of UV light. More on that later.

While trying to find a source of bulk UV glass glue at lower cost I came across a material called Liquid Optically Clear Adhesive (LOCA), the name of which immediately gave me a clue that it might well be useful to look at. LOCA is used in industry to glue the glass protective screens onto the LCD panels in smartphones and tablets and has been around for some time now. However, we can thank the likes of Apple for producing such fragile screens, and clumsy users who break them, for its current widespread availability. As with any high tech industry a host of service industries have sprung up to take advantage of the market and one of these is the supply of tools and materials to effect DIY repairs, including the repair of cracked phone screens. LOCA is now sold either by itself or as part of a kit including tools and a debonding solution, although reports tend to indicate that the debonding solution may have doubtful effectiveness. All we require for slide mounting is the LOCA itself and it is available from a number of sources.

I have used Aliexpress – the Chinese equivalent of Amazon, but with much longer delivery times – for several years and so far have not been let down much in any of my purchases, so it was on their website that I first searched for LOCA. Unsurprisingly, I found any number of sellers offering to supply it, but it was the price that took me aback - £4 for a 50g tube, or £8.50 including an applicator gun. This included postage from China and in the event delivery took a mere six days. Buy it in larger quantities and the price goes down still further, my latest purchase was for 5x50g tubes at under £15.

LOCA comes in at least two variants, TP-2500 and TP-1000, the numbers apparently referring to the viscosity in mPa.s, but it also appears that TP-1000 has a higher adhesive strength. I initially bought TP-2500 but I have since purchased a tube of TP-1000 as well, at a very similar price. It's difficult to pin down the precise specifications of LOCA, there are minor variations between suppliers but those shown below are typical;
Type: TP-2500F
Adhesive: acrylic resin
Curing: UV waves of 250-400nm
Transmission:> 99.8%
Viscosity (Brookfield RVT measurement, 25C): 2500mPa.s
Hardness (ASTM D2240): 18-30
Curing conditions (25C): 2300-2800 mJ/cm2
Refractive index (Abbe at 25C): 1.51

Shrinkage: <1%

Yellowness index (ASTM D1925/C2): (1.0>) 0.2

Properties: high bond strength, non-yellowing, high adhesion, water resistance

Recommended curing of 10-15 min (on both sides of the display)

It's equally difficult to pin down any definitive safety information on LOCA, the manufacturers of this material appear to keep most of the details hidden from public access and I have been unable to obtain any safety data that I would regard as absolutely reliable. However, as LOCA does not contain a high percentage of volatile components it seems likely that it is safe to use in the sort of quantities we would employ provided you do not allow skin contact. Most of the UV setting methacrylate adhesives contain Acrylic acid and Methacrylic acid, both of these may cause skin irritation and apparently if inhaled in large enough doses may cause pulmonary oedema, but the percentage of the acids in LOCA is reported as low and they are not volatile.

The issue of materials safety is a tricky one as it can be difficult to interpret any data you can find and as I've found with LOCA, manufacturers may not be keen to divulge the exact composition of their products. My personal preference is to follow what I would regard as a reasonable precautionary approach and I have made the personal decision to treat LOCA with respect, I wear gloves while using it and work either in a well ventilated space or, preferably, use an extraction hood. I've given details of a low cost, very effective extraction hood at the end of this article.

LOCA comes in tubes of various sizes, I would recommend buying either the 30g or 50g versions as they are very cheap and one tube gives more than enough to experiment with. In fact, if we work on the basis that a slide requires three drops of mountant then at roughly 50ul per drop this means we can potentially produce 7 slides per ml of mountant, or over 300 slides per 50g tube. My bulk purchase of 5 tubes means that the cost of the mountant has now dropped to 1p per slide.

It is also possible to buy a dispensing gun, normally used for applying LOCA to smartphones or tablets, but I have found that these can be tricky to use. I have resorted to using a short length of 20mm plastic electrical conduit, which fits exactly into the LOCA tube rather like the plunger on a syringe, and is available at DIY stores for approximately £1.20 for a 2m length. LOCA is sometimes quoted as having a shelf life of 6 months, I would recommend keeping the tubes in a dark drawer to keep the ambient UV as low as possible.

Having assembled all of the materials and equipment, I have now used LOCA to make a number of slides and have found it to be remarkably effective and simple to use, with some interesting properties. So far I have mounted Radiolaria, insect wings, microfossils from chalk and Gault clay,

pollen both stained and unstained, and thin sections of plant material. Initially I had problems with small bubbles being included in both the Radiolaria and insect wing slides, but I've had this problem before with other mountants and my personal experience has been that this is caused by the mountant failing to properly wet the specimens. In order to avoid bubbles the specimens need to have a thin layer of a solvent that is miscible with LOCA and I have found that suitable liquids include, xylene, butyl acetate, ethyl acetate, xylene and d-limonene (Histoclear). My original intention was to avoid the use of xylene so that option has been discarded; both of the acetates have a strong odour so I have now settled on d-limonene which has the distinct advantages of being a reasonably safe product and a smell of oranges. The latter property means that it is definitely domestically friendly. In the case of thin tissue sections the clearing agent at the end of processing is normally either xylene or Histoclear so no changes to the usual protocol are necessary.

To produce a good Radiolaria slide I place a very small drop of d-limonene on a clean slide and add a small quantity of the Radiolaria sample, which resembles a fine dust. The slide is allowed to dry off for a couple of minutes and I then add a tiny quantity of the LOCA and examine the slide under a stereo microscope. This is where the UV nail varnish comes in handy because I don't want the LOCA to set too quickly as this stage. I have made a filter to fit on the incident light system that consists of a glass disk coated with several layers of UV nail varnish, this absorbs any UV light given off by the incandescent bulb and by closing the room curtains I can minimise the chances of the LOCA being set by ambient UV.

I spread out and arrange the Radiolara in the LOCA and by also spreading out the mountant I make it as thin a layer as possible. Any bubbles can be removed by the use of a capillary tube or mounted needle and exposure to a few seconds of UV light sets the LOCA. The Radiolaria are now held in position in the centre of the slide, the addition of a couple of drops of LOCA and the coverslip will not move them. Adding too much LOCA is not a problem, simply let this flow out of the edges of the coverslip and then set using UV light for around two minutes.

The next stage involves cleaning off the excess and will probably be regarded with horror at first but it represents the major advantage of the mountant. When LOCA has set the excess around the edges still has an oily coating on top, just ignore this and use a detergent and hot water to remove it. LOCA does not set hard, it turns to a firm gel that can be removed cleanly and without damaging the coverslip. My preference is to spray the slide with Cillit Bang degreaser, scrub it with an old toothbrush – and I mean really scrub it – and wash off in hot water. If you tried this with just about any other mountant within a few minutes of making the slide, the coverslip would be at least disturbed and most likely would be scrubbed off. Not with LOCA, I can guarantee it will stay put and

most of the excess will be removed by the scrubbing action. Any that is left can be peeled off with a fingernail, just don't get the nail under the coverslip. That's it, side completed, dry it off and view.

So far I've just left the slides as is, I want to see if there is any shrinkage in the long term, but I'm intending to ring a few as well to see how much of a difference this makes to longevity. I've also tried some of the trickier mounting techniques that might expose any weaknesses, such as using LOCA to stick aluminium washers to slides to form cells, filling the cells with LOCA and microfossils and then coverslipping. The ones I've made so far have very few bubbles in them and the clarity is outstanding even though there is a 0.5mm thick layer of LOCA in the cell.

Overall, LOCA seems to have tremendous potential as a low cost, easy to use mountant but it needs more experimentation to optimise its use and much more testing to determine if it meets the longevity criterion. I would urge anyone who currently makes slides to give LOCA a go and report back the results; it's cheap enough to make this a viable exercise for anyone. Links to my purchase are given below but please note that the term "LOCA" is generic and covers a variety of formulations, products from different sellers may vary in composition or even the basic chemical type, even if the packaging is similar. This article is valid for the product I have used, if you find another one that performs better I'd be very interested to hear about it.

Postscript: Further experimentation indicates that cedarwood oil works very well as a "wetting agent" for LOCA and as this is a safe material and has a pleasant odour it's use is recommended. I have also been using plastic auto pipette tips attached to a rubber bulb to remove air bubbles with great success!

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