Ringing (Sealing) a microscope slide
and a comparison of ringing cements

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January 25, 2013

Abstract

Ringing a microscope slide improves its appearance as well as increasing its durability.

The properties of a number of commercial ringing cements were investigated, including a measurement of the thickness of ring.. All were suitable for use, though it is recommended that the final ring itself be protected with a layer of gold size.

Contents

1 Disclaimer

This document has been written in the hope it will be both informative and useful. To this end the author has tried to ensure the information included is accurate and reliable. However this publication is provided "as is", without a warranty of any kind. This document must not be assumed to be error free and the author will not be held liable for any loss, damage or injury arising from the information presented within.

2 Introduction

Ringing is traditionally one of the final steps in the creation of a permanent microscope slide. The term refers to the the process of applying a layer of sealing compound around the edge of a cover slip and beyond so it extends to the top of the slide. Once set this seal:

- Protects the coverslip from being accidental knocked off the slide due to rough treatment. This is especially relevant if the mountant is water based (glycerine jelly or an aqueous mountant) as, by their mature, such mountants are not very strong. Ringing is especially useful if the slide needs to be examined using an oil immersion objective. The oil must eventually be wiped off the coverslip and if this is not done gently the coverslip may be dislodged if it is not held in place by a ring.

- Whereas beauty is a very subjective matter, it is the author's opinion that a neat ring around the circumference of a cover slip greatly enhances the appearance of a slide. Also it is possible to use different colours of ringing cement to categorize slides (for example green rings for botanical subjects, red for animal histology etc).

- The ring hinders or prevents the continued evaporation of the solvent from the mountant and equally protects the mountant from attack by any solvents (water, xylene, immersion oil) which may get splashed on the surface of the slide. This is said to increase the longevity of a slide and again is particularly important where an aqueous mountant has been used.

- It prevents a coverslip sliding down a slide which has been stored for some years on its side. The author has never actually seen this happen, but has heard tales of glycerine mounts being affected when stored in a vertical position for a number of years.

Figure 1: Slide with crystallized resin mount

It is said that ringing is not needed for resin mounted slides where the mountant dries very hard. However the author has seen many examples of resin slides that do show deterioration after a number of years. One example is show in figure 1. This slide has a mount of a length of Orangutan hair and it was created in August 1987. The mountant was numount [1] . This slide was not protected by a sealing ring.
During the slide's twenty five life, the mountant has started to dry out and crack around the circumference of the coverslip. This cracking has intruded some 2mm into the mountant, and is visible as an orange band beneath the coverslip. For a close-up of the deterioration see figure 2. This shows the lower part of the coverslip and the band of cracked mountant.
It is not possible to say conclusively that this slide would have better survived the last couple of decades if it had been ringed. However, given the cracking took place where the mountant is in contact with the air, it is tempting to speculate ringing might have prevented or slowed this deterioration.
Ringing as a technique seems to be falling out of fashion, mainly for reasons of cost. It is normally applied to circular coverslips which are significantly more expensive that square coverslips of equivalent area. It needs to be applied using a good quality ringing table and these are becoming rare and represent an additional cost. Finally for commercial sides there is the additional manpower costs of applying rings and waiting for them to dry.

[1] A synthetic resin that does not acidify with time. It was marketed as an improvement to Canada Balsam.

Figure 2: Detail of crazed resin

3 How to Ring a Slide

For excellent instructions on ringing an side and indeed on all aspects of slide making the reader is referred to the paper "Slide Making" available at the Iceni Microscopy web site `http://www.icenimsg.co.uk/`.

4 A Comparison of Six Ringing Cements

The purpose of this paper was to compare a number of different ringing cements available to the author to see if they are all fit for purpose. The compounds used for the study were

- Brunseal Black
  Available from Brunel Microscopes (http://www.brunelmicroscopes.co.uk/).
  Use toluene for thinning the ringing cement and cleaning the brush.

- Brunseal Gold
  Available from Brunel Microscopes (http://www.brunelmicroscopes.co.uk/).
  Use toluene for thinning the ringing cement and cleaning the brush.

- An oil based Gold Size
  Available from art supply shops where it is used to attach metal leaf onto a surface. It comes with various drying time, the longer to dry the more expensive the gold size. Use the cheapest (and hence shortest drying time) oil based product available. Use white spirit (or xylene) for cleaning the brush after use. Gold size also comes as a water based product but this was not tried for this study.

- Humbrol Enamel paint
  The humbrol web-site is http://www.humbrol.com/. Humbrol do a range of enamel paints with a very wide range of colours. The author used a 14ml tin of Satin White purchased from a local model shop. Use white spirit for cleaning the brush.

- Gold size dyed with carmine
  Raw gold size is transparent when being applied to a slide and this makes its application difficult as one cannot see how much or how thick the final coat is. Hence the author coloured oil based gold size a red colour using powdered carmine. This was done immediately before the size was applied. A couple of brushes full of gold size were placed on a spare slide and a pinch of powdered carmine added (about twice the volume of a pin head). The whole was mixed with a brush and the result applied using the normal technique.

- Shellac
  This is a resinous secretion of an insect found in the forests of India and Thailand and is sold as dry flakes. The author has a bottle some decades old [2] which he still uses. The shellac is coloured purple and is dissolved in iso-propanol [3] which is used for thinning and cleaning the brush.

4.1 Ideal properties of ringing cement

Before comparing the different ringing compounds, let us consider what might be the properties of an ideal ringing cement and use these as a basis of comparison. A ringing cement should be:

- Impervious to the atmosphere
  The author did not have the equipment to evaluate this property and hence this was not a criteria in the following comparisons..

[2]Purchased from the company "Northern Biological Supplies" in the UK. However the company ceased trading on the death of the owner.

[3]Iso propyl alcohol, formula CH3.CH(OH).CH3

- Mechanically strong and rigid
  A primary aim of ringing is to prevent the coverslip moving or coming off the slide while it is being handled and possibly cleaned. Thus the ringing cement should resist being removed by rubbing.

- Solvent resistant
  Slides can easily be splashed (or cleaned) with water. If used with oil immersion the oil can find its way to the edge of a coverslip. Hence an ideal ringing cement must be insoluble in these liquids. Ideally the cement would also resist alcohol and xylene.

- Brightly coloured and opaque
  Ringing cements that are opaque can be used to cover imperfections at the edge of a coverslip [4]. Additionally a brightly and attractively coloured ring provides a professional look to a slide and different colours can be used to classify slides according to subject or to mountant. The author once ruined slides with glycerine jelly as mountant when he put them on a hot plate by mistake to 'set' along with resin mounted slides. To prevent such a mistake occurring in the future he now uses different coloured ringing cements for aqueous mount, fluid mount and resin mounted slides.

- Great Longevity
  The longevity of these ringing cements cannot be assessed in this study. Perhaps this will form the subject of a further paper in the distant future.

4.2 Experimental design

Each of the six ringing compounds was applied to a separate slide, that is one slide being allocated to each compound.
In each case a ring was applied in the centre of the slide using a ringing table and two coats of the compound, the second applied immediately after the first. In addition each slide was painted with three patches of ringing cement. The slide was thence left to dry for twenty-four hours.
The thickness of the central ring was measured (that is the distance from the surface of the slide to the top of the ring). This was effected by sprinkling lycopodium power [5] over the slide and the ring. Thence thickness could be

[4] Air bubbles, cracks in the edge of the coverslip etc

[5] A dry powder comprising the spores of club moss. This can be obtained from magical supply houses under the name of 'Dragonś Breath' and it is used by conjurers for pyrotechnic effects

determined by focusing a microscope on lycopodium powder on the top of the slide, noting the setting of the micrometer on the fine adjustment. Thence the slide was moved and the microscope focused on lycopodium powder on the top of the ring. Noting the change in the reading of the fine adjustment micrometer gave the thickness of the ring. Ten such readings were taken at various places around the circumference of the ring allowing a mean thickness to be obtained along with standard deviation.
The mechanical robustness and water resistance of the ringing cement was made by rubbing the ring with a damp tissue to simulate the wear that would be experienced in cleaning the coverslip. In this way the relative water and abrasion resistance of each of the cements could be assessed. Unfortunately this test was rather subjective and the resistance was classified as poor, good or excellent.
Finally, the resistance of each cement to immersion oil, iso-propanol and xylene was assessed by putting a drop of each of these solvents on the three painted areas respectively of each slide. Thence the solvent was removed by rubbing with a tissue and thus the resistance of the cement to each of the solvents was determined. Again this was a somewhat subjective test and resistance was classified as poor, good or excellent.
Finally the opacity of the pigments were determined. This was effected by painting stripes of brunseal black and a stripe of shellac down the length of a slide, both of these being well defined and colourful compounds. Once these were dry, single lines of each of the six compounds was painted across the width of the slide covering and at right angles to the first two lines. When these had dried, a dissection microscope was used to judge how opaque each ringing cements were by seeing how well they covered the lines of shellac and brunseal black. Again opacity was classified as poor, good or excellent.

4.3 Results

An evaluation of each of the six ringing cements follows below

4.3.1 brunseal black

Application
Easy to apply
Appearance
Forms a dull, matt black ring
Opacity
Good

Thickness of ring
Mean thickness 10.2 micron, standard deviation 5 micron.
Resistance to water and rubbing
Poor, the brunseal black could easily be rubbed off with a little water.
Resistance to immersion oil
Good.
Resistance to iso-propanol
Poor, removed by iso-propanol.
Resistance to xylene
Poor, removed by xylene.

4.3.2 brunseal gold

Application
Easy to apply
Appearance
Formed an attractive shiny yellow finish, albeit with a streaky finish.
Opacity
Excellent, though when applied as a ring one can see through the streaks.
Thickness of ring
Mean thickness 6.9 micron, standard deviation 5 micron.
Resistance to water and rubbing
Excellent, Reasonably resistant to rubbing and to water.
Resistance to immersion oil
Excellent..
Resistance to iso-propanol
Good, thought some 'bleeding' of yellow pigment.
Resistance to xylene
Poor, removed by xylene.

4.3.3 gold size

Application
A little difficult to use as it tended to 'blob' when applied via a ringing table (though this could be my poor technique). Since the gold size is transparent, it was difficult to judge how much material had been applied during the ringing process.
Appearance
Transparent, but the ring could be seen with care (and easily felt with a finger).

Opacity
Poor, ring is transparent.
Thickness of ring
Mean thickness 37.4 micron, standard deviation 6 micron
Resistance to water and rubbing
Excellent.
Resistance to immersion oil
Excellent.
Resistance to iso-propanol
Excellent.
Resistance to xylene
Poor, removed by xylene.

4.3.4 Humbrol paint

Application
Easy to apply.
Appearance
Forms a matt white ring.
Opacity
Excellent.
Thickness of ring
Mean thickness 17.4 micron, standard deviation 6 micron.
Resistance to water and rubbing
Excellent.
Resistance to immersion oil
Excellent.
Resistance to iso-propanol
Excellent.
Resistance to xylene
Poor, removed by xylene.

4.3.5 gold size stained with carmine

Application
More easy to apply than neat gold size as it is possible to see the ring going on the slide.
Appearance
An attractive shiny red coloured ring, though with a slightly granular appearance.

Opacity
Good.
Thickness of ring
Mean thickness 46.6 micron, standard deviation 11 micron.
Resistance to water and rubbing
Excellent.
Resistance to immersion oil
Excellent.
Resistance to iso-propanol
Good, though a little 'bleeding' of the red colour.
Resistance to xylene
Poor, removed by xylene.

4.3.6 shellac

Application
Very easy to apply and to get a thin neat ring. However this is probably due to my having more experience with this material that the other ringing compounds.
Appearance
A thin ring with an attractive shiny purple colour.
Opacity
Good.
Thickness of ring
Mean thickness 22.8 micron, standard deviation 8 micron.
Resistance to water and rubbing
Excellent.
Resistance to immersion oil
Excellent.
Resistance to iso-propanol
Poor, moved by iso-propanol.
Resistance to xylene
Poor, removed by xylene.
These results are summarized in a tabular form in figure 3.

5 Discussion

All ringing compounds were easy to apply and all gave some protection to the coverslip.

Ringing cement	Thickness	Resistance to			
		Water	Oil	Alcohol	Xylene
Brunseal black	10 μ	Poor	Good	Poor	Poor
Brunseal gold	7 μ	Excellent	Excellent	Good	Poor
Gold size	37 μ	Excellent	Excellent	Excellent	Poor
Humbrol	17 μ	Excellent	Excellent	Excellent	Poor
Red gold size	47 μ	Excellent	Excellent	Good	Poor
Shellac	23 μ	Excellent	Excellent	Poor	Poor

Figure 3: Result summary

The thicknesses of the rings were variable, gold size dyed with carmine being the thickest with good consistency. Hence this should be a good compound to use for building up a cell on a slide and might be expected to give best protection to the coverslip. The thinest ring was Brunseal gold. In addition to forming a thin ring, the thickness of Brunseal gold was very variable.
The ringing cements having the greatest resistance to solvents were gold size and Humbrol paint.
With the exception of neat gold size, all the ringing cements provided a good level of cover (that is good opacity). To the author's eye, the compounds Brunseal gold, Red gold size and Shellac were the most attractive as these had a shiny rather than a matt appearance.
A popular alternative to ringing cements is the use of nail varnish. The author did not include this in the study but suspects a coloured nail varnish might well have given results comparable to the best ringing compounds.

6 Suggestions for Further Work

Following on from this paper there are a couple of areas that could still be explored. These are

- Using the protocol developed in this paper, explore the properties of nail varnish and see how this compares to proprietary ringing cements.
- Test the colour fastness of the different ringing cements. This could be done by applying patches of different ringing cements to a slide and exposing it to direct sunlight (and possibly even rain) for a period of

months. The slide would then be compared with a control slide that had been stored in darkness, in order to see if sunlight bleaches the colour of the ringing cements.

7 Recommendations

Any of the ringing compounds tested above is an excellent choice for ringing a slide. However the recommendation would be to apply two rings, that is one of the chosen ringing compound, let this dry and thence apply a second ring of neat gold size. The latter will increase the thickness of the ring and provide an additional level of protection against exposure to solvents.