Realgar mounted slides for the imaging of diatoms

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Summary

Various techniques are used in diatom microscopy to enhance their appearance. Some of these, such as Differential Interference Contrast, rely on modification of the microscope equipment itself, while others look to the mounting technique, for example the use of high refractive index mountants. In 2023 I wrote an article for the Quekett Journal of Microscopy on the use of metal and metal oxide coatings for diatoms to help improve their visibility. This new article looks at another approach for improving diatom visibility, the use of Realgar (Arsenic Sulphide) as a high refractive index mounting medium. The classical spelling of Sulphide is used here, although the spelling Sulfide is often used today. The mineral Realgar is a very specific form of Arsenic Sulphide (α-As_4S_4), but this is not the same as that used on slides. As we shall see with regards to its use as a microscope slide mountant, a number of makers have produced Arsenic Sulphide (and indeed Arsenic Selenide) based mountants. Throughout the article, the term 'Realgar slides' is used to cover Arsenic Sulphide based mounts. An Arsenic Selenide mounted slide is also included as it is relevant to the discussion. A range of slides from different time periods and makers will be shown along with images from them. The challenges and advantages of this type of approach will be discussed, as well as potential drawbacks with its use.

Introduction

How a sample is mounted hugely impacts its appearance when performing microscopy. As discussed in a previous article looking at high refractive index (RI) metal and metal oxide based mountants, increasing the RI of the mountant improves the contrast and visibility of diatoms [1]. The RI of the silica in diatoms has been reported as 1.434 [1]. High RI mountants such as Hyrax, Naphrax and Sirax were therefore often used for mounting diatoms, as these provided increased contrast and helped with visibility of the subject. However materials with even higher RI's (above 2.0), such as Realgar have also been used as mountants for imaging diatoms. This article will summarize the author's findings to date on Realgar based mountants for the preparation of diatom slides. All of the microscope images shared here were taken using a modified Olympus BHB microscope and are transmission images. The Realgar based slides currently in the author's collection are shown in Fig. 1.

Early Realgar slides

The use of Realgar as a mountant for diatoms started in the late 1800s. In his 1893 book [2] Henri Van Heurck wrote: '*In 1884 our friend, Professor H.L. Smith, made the important discovery of his highly refractive yellow medium. We have good reason for calling this discovery 'important', however, to fully appreciate the service rendered to diatomists by the American savant, one must be making a study of diatoms and have realized how difficult, I should say almost impossible, it was to examine the striation of the most minute forms.*

The yellow medium of Professor Smith consists of realgar dissolved in Bromide of Arsenic. It is not quite the same as the article known as realgar in commerce, which is a substance as brittle as glass, brownish in colour and is perfectly

Figure 1a. Slides, from left to right: (1) Dr Henri Van Heurck. Mélange including *Pleurosigma angulatum, Surirella gemma, Van Heurckia crassinervis* and *Amphipleura pellucida.* (2) Dr Henri Van Heurck. *Amphipleura pellucida.* (3) Henry Joseph Grayson, Melbourne. *Van Heurckia* (?) Ballarat, Victoria in pure Realgar, refractive index 2.549. (4) Eric Doddrell Evens. *Amphipleura pellucida.* 19/2/1941. Coverglass 0.0064 inch; specimens from Thum. In Realgar 1pt, sulphur 1.5pt. (5) Eric Doddrell Evens. *Pleurosigma, Nitzschia,* 17/5/1932. Coverglass 0.007 inch. Butcombe, near Bristol. In Realgar 1pt, sulphur 1.7pt. (6) *Pleurosigma angulatum.* 31/10/1935. In Realgar.

Figure 1b. Slides, from left to right: (7) Samuel Henry Meakin. *Amphipleura pellucida.* December 1937. In Realgar, arsenic 7 parts, sulphur 23 parts. Refractive index approximately 2.4. (8) *Amphipleura pellucida, Frustulia saxonica.* In Realgar. (9) Watson and Sons Ltd. *Nitzschia singalensis.* In Realgar. (10) C. Baker, E. Thum. Test, mélange, small diatoms. Coverslip 0.16mm. In Realgar. (11) C. Baker. *Amphipleura, Navicula, Pleurosigma, Surirella.* Test diatoms in realgar. (12) C. Baker. *Frustulia saxonica.* Diatomaceæ. In realgar.

Figure 1c. Slides, from left to right: (13) Watson and Sons Ltd. *Pleurosigma angulatum, Pleurosigma balticum,* and *Surirella gemma.* In Realgar. (14) H.C. Wheeler, Montreal, Canada (no makers name on the slide). Diatomaceæ, stomach of fish from China. Dated 4/3/1921(?). In resublimed Realgar. (15) *Amphipleura pellucida.* Coverslip 0.16-0.17mm. In Realgar. (16) Watson and Sons Ltd. *Amphipleura pellucida.* In Realgar. (17) Watson and Sons Ltd. *Coscinodiscus asteromphalus* arrangement. In a specially high refractive preparation of Realgar. (18) *Amphipleura pellucida.* In Arsenic Selenide, As_2Se_{10}, and Clearax.

transparent. When Professor H.L. Smith acquainted us with the formula of this medium, he produced this realgar by melting two parts of Sulphur with one part of metallic arsenic, and fusing the mass at a red heat for several hours. When we had made the realgar in this manner, we found out after a few trials that the product could be obtained more easily, and at the same time in a purer state, by fusing together in a retort one part of Sulphur and 1.7 parts of Arsenious acid, and by raising the temperature to a point at which the product distills. Realgar thus obtained by distillation has quite the appearance of the natural realgar of mineralogists.'

Van Heurck then goes on further to describe the process he used to make Realgar for slides and the rest of the section is well worth reading. Examples of slides offered by Van Heurck are shown in Fig. 1a, slides 1 and 2, along with images from them in Fig. 2. It should be noted that Allen Y. Moore is credited with independently discovering the value of Realgar for mounting diatoms, but was unable to make satisfactory mounts with it due to its high melting point [3]. Henry Joseph Grayson was also an early experimenter with Realgar for mounting diatoms and reports of his work are given in *Nature* around 1900 [5-7]. A slide by Grayson is shown in Fig. 1a, slide 3 and an image from this slide is given in Fig. 3.

Other makers of Realgar slides

A wide range of slide makers, both companies and individuals, have produced Realgar slides including Watson and Sons Ltd., Baker, Meakin, Thum, Wheeler and Evens.

Example images from a range of these slides are given in Figs. 4a-4d. Klaus Kemp also prepared some Realgar slides, an example of which is shown in Fig. 5, along with images of two diatoms from the slide. Almost all of the Realgar slides are strews, however one of the slides in the author's collection is a cross shaped arrangement of *Coscinodiscus asteromphalus* by Watson and Sons

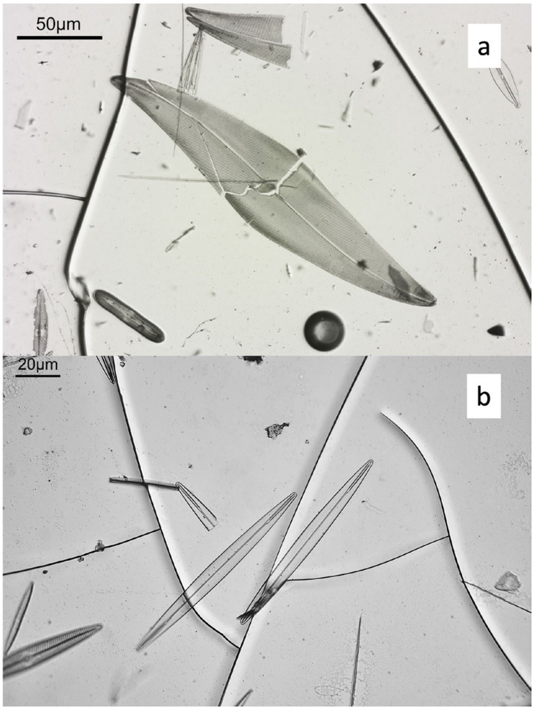

Figure 2. Van Heurck slides: a. slide (1) in Figure 1a. White LED light. 40x Olympus Dplan Apo UV NA 0.85 objective, Olympus Aplanat Achromat condenser with oblique lighting, Olympus 2.5x NFK photo eyepiece, monochrome converted Nikon d800 camera. Single image (no stacking). b. Slide (2) in Figure 1a. 450nm LED light, 20x Nikon Plan Apo NA 0.65 objective, Olympus Aplanat Achromat condenser, brightfield lighting, 2.5x Nikon CF PL photo eyepiece, monochrome converted Nikon d850 camera. Single image (no stacking).

Ltd (Fig. 6.) This arrangement will be discussed further later in the article.

Arsenic Selenide

While most of the work done in this area seems to have concentrated on Arsenic Sulphide, it has been reported that Arsensic Selenide can also be used as a mountant, and that this has the potential to provide an even higher RI than Realgar: '…*objects coated with arsenic selenide, which is ordinarily opaque, are as visible as in a balsam mount, but the resolution obtainable is nearly double because the index of refraction of the selenide is nearly 3.*' [8]. Example slides using Arsenic Selenide seem to be extremely rare, and the author has so far only found one of them by an unknown mounter (Fig. 7).

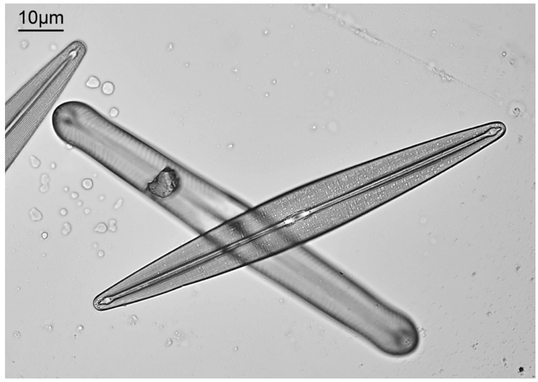

Figure 3. Henry Joseph Grayson slide, number (3) in Figure 1a. 450nm LED light, 63x Leitz Pl Apo NA 1.40 objective, oil immersion. Olympus Aplanat Achromat condenser, oil immersion, brightfield lighting, 2.5x Nikon CF PL photoeyepiece, monochrome converted Nikon d850 camera. Single image (no stacking).

Discussion

The mineral Realgar, a specific form of Arsenic Sulphide (α-As_4S_4) occurs in nature as a glassy looking, soft red mineral, an example of which is shown in Fig. 8. Although as far as the author is aware, chemically, Realgar mountants are not identical to the mineral Realgar, the use of the name Realgar comes from the use of Arsenic and Sulphur based chemicals to produce it and the similarity in appearance to the mineral [2]. The complexities of and methodologies for preparing and using this material are discussed in a number of articles [2,8,9] with a practical summary of different approaches given in [10]. It should be emphasized that this is not a simple, or safe, material to prepare and handle, so why go to the effort of using it? It is because of the high

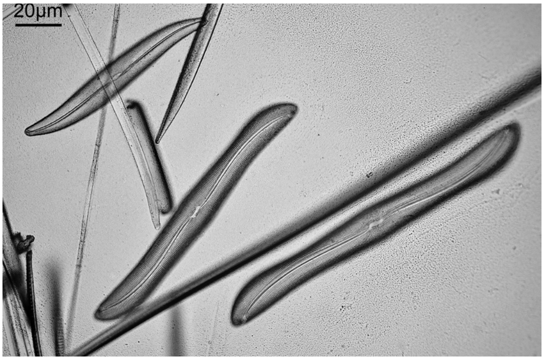

Fig. 4a. H.C. Wheeler slide, number (14) in Figure 1c. 450nm LED light, 63x Leitz Pl Apo NA 1.40 objective, oil immersion, Olympus Aplanat Achromat condenser with oil immersion, brightfield lighting. 2.5x Nikon CF PL photoeyepiece, monochrome converted Nikon d850 camera. Single image (no stacking).

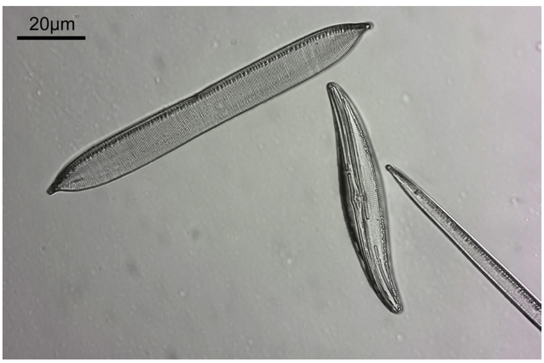

Fig. 4b E.D. Evens slide, number (5) in Figure 1a. 450nm LED light, 60x Olympus Splan Apo NA 1.40 objective, oil immersion, Olympus Aplanat Achromat condenser with oil immersion, oblique lighting, 3.3x Olympus NFK photoeyepiece, monochrome converted Nikon D800 camera. Stacked image (Zerene, DMap).

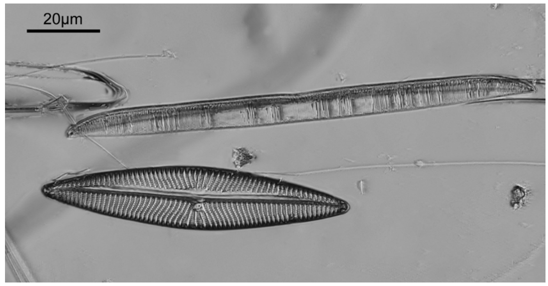

Fig 4c Watson and Sons Ltd slide, number (9) in Figure 1b. 450nm LED light, 60x Olympus Splan Apo NA 1.40 objective, oil immersion, Olympus Aplanat Achromat condenser with oil immersion, oblique lighting, 3.3x Olympus NFK photoeyepiece. Monochrome converted Nikon D800 camera. Stacked image (Zerene, DMap).

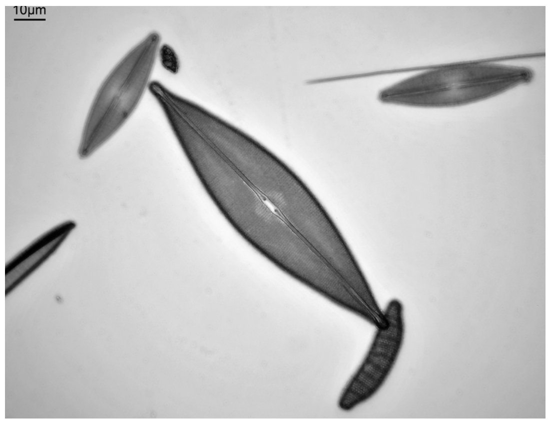

Fig. 4d C. Baker slide, number (10) in Figure 1b. 450nm LED light, 63x Leitz Pl Apo NA 1.40 objective, oil immersion, Olympus Aplanat Achromat condenser with oil immersion, brightfield lighting, 2.5x Nikon CF PL photoeyepiece. Monochrome converted Nikon d850 camera. Single image (no stacking).

RI that is possible with it – typically reported as being up to around 2.4 [11]. Diatom silica has an RI of 1.434, and the effectiveness of differences in the RI between the subject and the mountant can be assigned a 'visibility index', where essentially the larger the difference the more visible the subject becomes [12]. This correlates well with the authors experience of viewing these slides which showed high contrast even under simple brightfield illumination, and explains why Realgar mounts were desirable for improving diatom visibility.

While improved contrast and visibility is certainly an advantage for using this material as a mountant, there are unfortunately some quite severe issues and challenges with it as well which will now be discussed. First to consider is the challenge of making the material and the toxicity of the ingredients. Arsenic is a poison

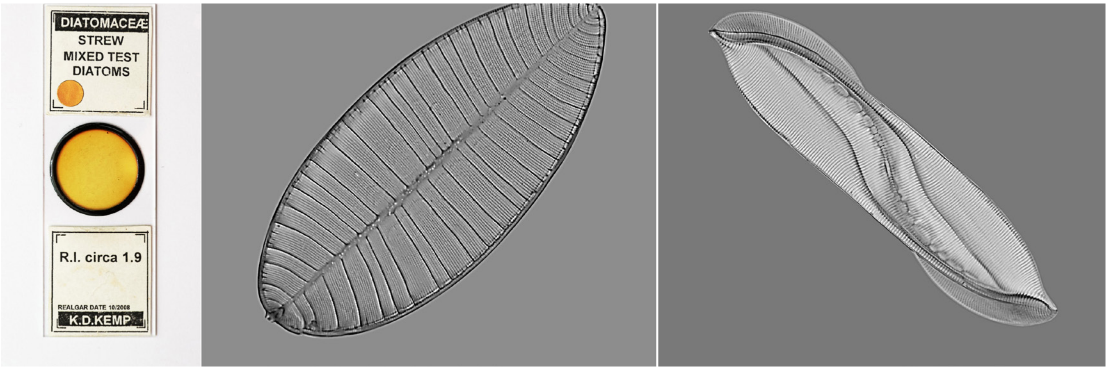

Fig. 5. Left: Klaus Kemp Realgar microscope slide (strew, mixed test diatoms, RI circa 1.9). Centre: *Surirella gemma*. Right: *Entomoneis* sp. (perhaps E. *alata*). Diatom images captured using brightfield with double immersion and 550nm lighting with an IF550 filter, and a 60x Plan Apo objective on an Olympus microscope. Diatom and slide images provided by Daniel Han.

and exposure can lead to vomiting, diarrhea, abdominal pain, skin pigmentary changes and in extreme cases death. Handling the raw materials should not be done without knowing the correct procedures for doing so. Making the Realgar and coating the diatoms involves using heat to sublime the Realgar onto the sample and (with some approaches) the use of a vacuum. In 1939, Samuel Henry Meakin wrote with regards to Realgar; '*As this medium must be used in the plastic state, only strewn slides can be made.*' [13]. While it is true that the majority of slides seem to be strews, the author does have one which is an arrangement (slide 17 in Fig. 1c). At this point it is unclear how Watson and Sons Ltd produced this or whether it is a one off, as the author has found no other examples of Realgar mounted arrangements. Once prepared the slides need to adequately sealed around the coverslip. Van Heurck described that preparations of the 'arsenical medium' are very liable to undergo alteration after making [2], and that to preserve them the following precautions must be observed;

"*1. The preparations should be made in very dry weather, in the open air and in the sun.*

2. The arsenical medium should be heated until it cracks more or less, when cooling.

3. The preparations should be made very quickly; the covers and slides should be warm just when the medium is applied.

4. As soon as the preparation is completed, and while it is still lukewarm, a thick layer of gum lac should be applied around the edge of the coverglass."

As discussed by Van Heurck, excluding water from these slides was an important factor in their production. Indeed, one of the slides in the authors collection has quite severe damage to the ring around the coverslip and the Realgar does look to have degraded starting from this point (Fig. 9), remaining clear at the position furthest away from the damaged coverslip ring.

There is little recent information on the preparation and use of Realgar [9]. However Klaus Kemp did produce Realgar slides as recently as 2008. In communication with Bill Dailey in 2008 about a jar of RI=1.9 material that he had in his possession, Klaus wrote with regards to its use; '*Be assured that I took your advice and dealt with this in the strictest safety conditions. I found that when I tried to break it into pieces with a metal rod, the rod penetrated a thin crust and went into a thick tar consistency, some adhered to the rod, which*

I then smeared a small amount on to the slide, the Diatoms were strewn on the cover and laid on top of the Realgar, this was all done in a well ventilated Conservatory, the slide was then place over a small spirit burner outside of the conservatory, as there was only a slight breeze West to East I stood upwind and melted the Realgar, while still slightly warm I put a shellac ring round the cover. I have to say the slide looks just great and I do not overstate the resolution it gives SUPERB. I just so loved being able to look at so many Diatoms with a completely different feel to them now that I can see so much structure." [14].

The material Klaus had was likely one based on $As_2S_3/AsBr_3/(C_6H_5)_3As/S_8$ as described in [9] which was reported to have an RI of 1.85.

The deep yellow colour of the Realgar slides makes it difficult, or in some cases impossible, to use blue light for imaging. The author often uses a filtered 450nm LED light source for imaging diatoms and with these slides long exposure times were needed due to the amount of light being absorbed by them. This can be demonstrated by examining the transmission spectra, as measured using an Ocean Optics FX spectrometer and deuterium/halogen light source, of a number of the slides presented here (Fig. 10). As this figure demonstrates, transmission of light through the Realgar slides is very low in the blue region around 450nm. For the Arsenic Selenide this even more extreme, and it is essentially opaque until above 500nm. This restricts the microscopist to the use of longer wavelengths for imaging these types of slides, which is of course an issue when it comes to achievable resolution – as wavelength increases, then for a given numerical aperture of the objective the resolution will get worse.

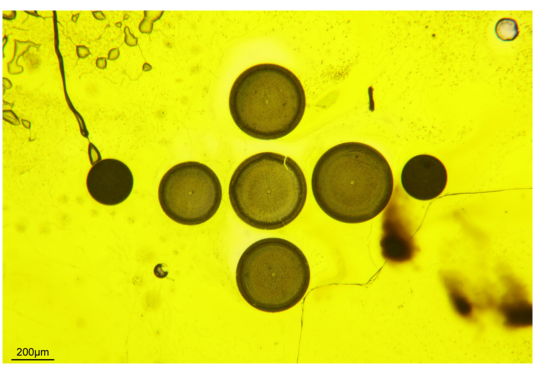

Figure 6. Watson and Sons Ltd arrangement of *Coscinodiscus asteromphalus*. Slide (17) in Figure 1c. White LED light, 4x Zeiss Planapo NA 0.16 objective, Olympus Abbe condenser with top lens removed, 2.5x Nikon CF PL photoeyepiece. Canon EOS 5DSR camera with auto white balance. Single image (no stacking).

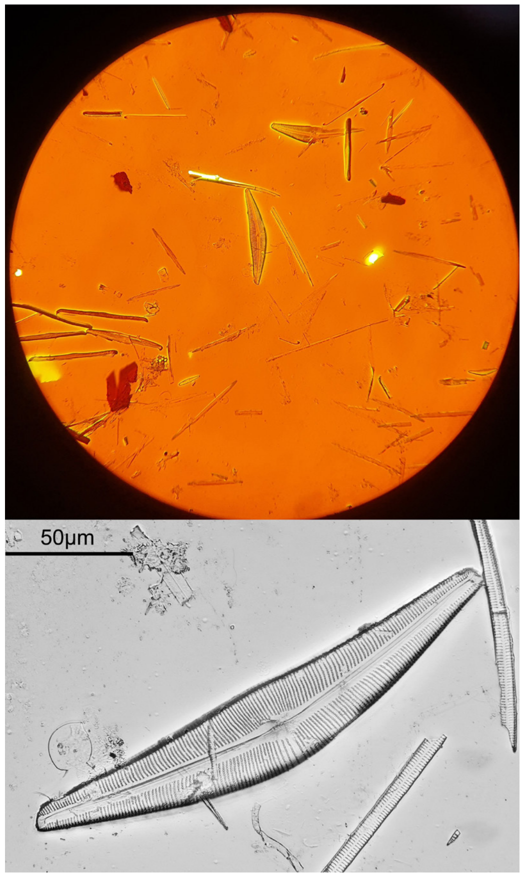

Figure 7. Arsenic Selenide slide, number (18) in Figure 1c. Top: view through the eyepiece of the microscope using a camera phone. Bottom: white LED light, 60x Olympus Splan Apo NA 1.4 objective with oil immersion, Olympus Aplanat Achromat condenser with oil immersion, brightfield lighting, 2.5x Olympus NFK photoeyepiece. Monochrome converted Nikon D800 camera. Stacked image (Zerene, Pmax).

Figure 8. An example of the natural mineral Realgar, approximately 3cm across.

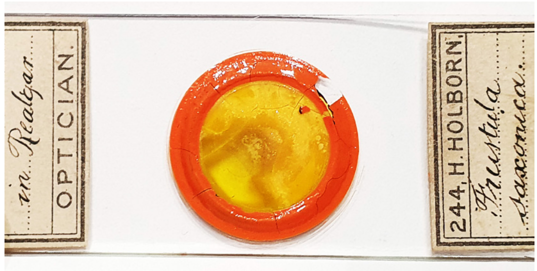

Figure 9. Closeup image of slide (12) from Figure 1b showing the damaged coverslip ring and the degradation of the mountant in the nearby areas of the slide.

Cracks tend to occur quite frequently in Realgar mounts, and they are visible in many of the author's slides. This is not unusual with films deposited in the way as they are produced due to the stresses in them. Although not shown here in great detail, the Realgar films tend to be quite heterogeneous. The author has seen areas within a slide which look different at 450nm with respect to white light, indicating potential variations in chemical composition throughout the mount. If the opportunity arises at a future date, the author would like to examine these further using different analytical techniques to better understand what the 'Realgar' mountants actually are chemically.

High RI (or more precisely large differences in RI between different parts of the optical train) brings with it the problem of spherical aberrations which can impact resolution and reduce image quality [15,16]. This is something that will occur for all mountants where the RI is different to those of the glass of the slide, coverslip and optics, and the immersion oil (if being used).

One final, but rather serious issue with Realgar mounts is that they are presumably light sensitive (assuming they behave in a similar manner to the mineral Realgar). This is obviously not ideal for a microscope slide mountant. On exposure to light the Realgar forms other sulphides and oxides of Arsenic, and the degradation seems to occur most quickly with light between 500nm and 600nm [17]. For anyone with these slides, storing them in the dark would be strongly recommended as would minimizing the time they are exposed to the lamp for imaging.

Is there really a need for Realgar mounts these days? Meakin nicely summarized Realgar in a 1939 article; '*Realgar is the most difficult [mounting] medium to use. It is only because of its high refractive index that it is required. The mounter will have the prepare his own Realgar, and unless he has some knowledge of chemistry he should not attempt to do so, because the entire process is dangerous.*' [13]. With the advent of mountants such as Naphrax, Pleurax, Hyrax and Sirax which have higher RI's than Styrax, diatom slides with improved contrast can be prepared, reducing the advantages of Realgar. However these have not reached the extremely high RI of Realgar, and some of these organic mountants are no longer available. It should be noted that titanium oxide based mountants also produce high RI, high contrast slides [1]. However they too come with significant challenges when preparing them. The development of imaging techniques such as polarization, differential interference contrast and phase contrast has also enabled higher contrast images to be captured using more

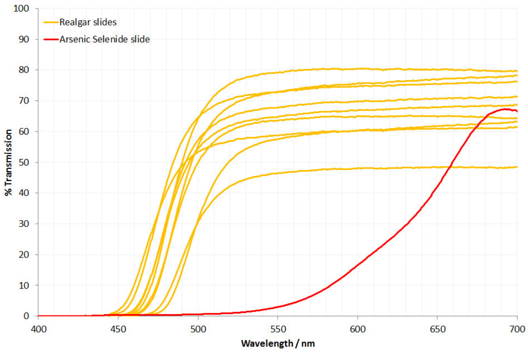

Figure 10. Transmission spectra between 400nm and 700nm through a range of Realgar slides and an Arsenic Selenide slide.

conventional organic based mountants, obviating the need for these ultra-high RI mountants. As such Realgar mountants are less needed today than when they were developed, however they provide slides with a very distinctive look, and do enable high contrast images to be taken with very simple brightfield lighting. As such they will no doubt continue to be of interest to those with a passion for the science and history of microscopy.

Conclusions

Mounting diatoms in high RI materials such as Realgar has been carried out for over 120 years to help improve their visibility for microscopy. The resulting slides provide unique and beautiful diatom images, giving a marked improvement in contrast when compared with conventional lower RI mountants. The process of making slides with this mountant raises considerable challenges and at this stage the author is not aware of anyone who is still doing this process, commercially at least. With the advent of newer imaging approaches for improving resolution and contrast, historical approaches like this have fallen into disuse, however they remain an interesting and fascinating addition to the world of microscopy and a testament to the ingenuity of the early microscopists. I will conclude this article with the following excerpt from a 1940 article by Hanna and Grant; '*A famous diatomist told one of us at the beginning of his career that he had a cemetery of spoiled slides representing wasted effort in search of a better mounting medium than styrax and strongly advised against our trying anything else. No words could have inspired a greater urge to investigate the high-index materials.*' [8].

Acknowledgements

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