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CASTING, REPLICATION, AND ANAGLYPH STEREO IMAGING OF MICROSCOPIC DETAIL IN FOSSILS, WITH EXAMPLES FROM CONODONTS AND OTHER JAWLESS VERTEBRATES

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ABSTRACT

Sophisticated techniques, such as computed tomography and scanning light microscopy, now allow palaeontologists to image the microscopic details of fossils even when scanning electron microscopy cannot be used. Occasionally these techniques are not always applicable, and where this is the case, methods involving fossil replication offer an alternative. I describe here a series of techniques for moulding, casting, imaging, and three-dimensional illustration of microfossils (or microscopic details of larger fossils). For moulding fossils (or casting mouldic specimens), room temperature vulcanizing silicon rubber provides a strong and flexible medium with low levels of shrinkage. RTV rubbers are also capable of replicating microscopic details of only a few micrometres. Similarly, epoxy resins are rigid, durable, and long lasting. In combination with RTV silicon rubber moulds, epoxy casts offer higher levels of resolution than any other medium. Details of specific RTV rubbers and epoxy resins that are widely available and work well with small fossils are provided.

For imaging, the advantages of stereophotography to illustrate fossils have long been appreciated, but the use of stereo-pairs is limited by their maximum size (generally only 5 or 6 centimetres). With the widespread availability of powerful image editing software, it is now a straightforward matter to produce anaglyph stereo images of any size. I provide step-by-step instructions and a set of actions to automate the process in Adobe Photoshop®. Anaglyph stereo images can be extremely useful research tools in their own right, but combined with electronic communication and publication they offer a simple and inexpensive means for illustrating fossils, and their microscopic details, in three-dimensions.

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INTRODUCTION

Despite advances in recent years, microfossils, or small details on larger fossils, can be difficult to image. Advances include: equipment that allows routine scanning electron microscopy without the need to coat specimens with conductive materials; techniques such as scanning light microscopy (Scott et al. 2000) and extended focus image montage methods (e.g., Holbourn and Henderson 2002; Knappertsbusch 2002) that are able to overcome the depth of field limitations of optical photomicrography; high resolution colour laser scanning (Lyons et al. 2000) and computed tomography (Rowe 1996; Brochu 2000). These techniques and methods are increasingly applied to palaeontological problems, yet there are still instances when availability of equipment, cost considerations, or the nature of the material under investigation mean that direct imaging using these techniques is impractical or impossible. I describe here a series of simple and inexpensive methods for specimen preparation and replication that allow for high resolution imaging of microscopic details in such instances. I also describe a simple and rapid process for producing anaglyph stereo images, and provide a set of Adobe Photoshop® actions for partial automation of this process. Except in some details, none of these techniques is new. The purpose of this paper is to highlight their usefulness in palaeontological research and communication, especially in dealing with microscopic subject matter, to provide specific details of methods and widely available materials that have been found to work well, and to illustrate with examples from conodonts and other jawless vertebrates how they can reveal important detail that is otherwise unobtainable. It is worth emphasizing that before applying any of these methods to borrowed material, full permission should obtained.

Silicon Rubber Moulding

For most palaeontological applications, the choice of moulding material is between latex-and silicon-based compounds. Latex rubber solution is still in common use by palaeontologists and is suitable for many purposes, but it has the major disadvantage of shrinkage (Goodwin and Chaney 1994). Silicon-based materials, especially room temperature vulcanising (RTV) silicon rubbers, on the other hand, have lower levels of shrinkage and also offer other advantages over latex, including strength, flexibility, (Goodwin and Chaney 1994), and, once cured, good shelf-life stability (although some are susceptible to degradation by ozone and UK light, so storage in a dark, anoxic environment will maximise shelf life). Silicon rubbers also have the capability of capturing details at the submicrometre level (Rose 1983). Latex moulds have poor resistance to epoxy and polyester resins, and a parting agent must be used to protect the mould (Goodwin and Chaney 1994). Consequently, methods using latex moulds with resin cannot replicate microscopic details.

The use of room temperature vulcanizing silicon rubbers (and epoxy casting – see below) in palaeontology was pioneered by Waters and Savage (1971) and is now widespread. Waters and Savage (1971) and more recent publications (e.g., Reser 1981; Chaney 1989; Goodwin and Chaney 1994) cover the general methodology of mould making and contain details of precautions that will ensure that use of RTV silicon rubbers causes no damage to either the fossil or the humans involved. The focus of this paper, however, is moulding and casting microscopic detail, and although Waters and Savage (1971), for example, replicated micromammal remains down to a few millimetres in size, the use of silicon rubber to mould smaller fossils, including microfossils, has been limited. Chaney (1989) attempted to mould forams, but had only partial success, whereas Siveter (1984), obtained good results using RTV rubber to cast the fine details of ostracods preserved as natural moulds. Some of the methods presented here have developed from those of Siveter (1984).

The basic steps of the process as I have applied it are as follows. Prior to moulding, the surface of the specimen is coated with a thin layer of separator (or consolidant) or a release agent. This layer serves several purposes: it prevents loose parts of the specimen from being pulled off when the cured mould is removed; it prevents silicon fluid from penetrating the surface of the specimen and leaving a dark stain (particularly important if the specimen preserves traces of soft tissue remains); and it improves the chances of removing the cured mould cleanly from the specimen without leaving torn-off fragments of rubber in undercuts and the deeper recesses of the specimen. A solution of pvb [a terpolymer of poly(vinylbutyral), poly(vinyl alcohol), and poly(vinyl acetate)] in methanol has been found to be an effective consolidant and separator, which has the advantages of being safe, stable, and reversible (Elder et al. 1997). The solution is easily prepared to the right consistency to allow good impregnation of the surface without overglazing and obscuring morphological details. If applied too thickly, excess consolidant can be removed or redistributed by brushing methanol over the surface. Determining how much separator is required to protect the specimen without obscuring detail is a matter of judgement and experience, but if patches of the dried separator on the surface of the specimen appear smooth and shiny, it is probably too thick. Chaney (1989) suggested that use of a separator prevents the mould from picking up microscopic detail, but I have found that if the coating is thin enough, the mould can pick up details down to a few tens of micrometres or less (see examples below, especially example 3). Nevertheless, if the specimen will allow it, maximum detail will be obtained if no separator is used. It is worth noting here that in selecting a separator, reversibility is a particular important property. Materials with questionable or limited reversibility should be avoided as they may be difficult to remove from the specimen.

A release agent may be used instead of, or in addition to, a consolidant or separator. In itself this release agent will do little to stabilise the surface of the specimen, but it will reduce or prevent penetration of silicon fluid and facilitate clean removal of the cured mould. A 50% solution of domestic dishwashing detergent (washing-up liquid) in water makes an effective release agent that does not obscure surface details. A thin coat can be painted onto the specimen with a soft brush and left to dry before moulding.

In most cases, the area of the specimen to be moulded will need to be surrounded by a wall to prevent silicon rubber from flowing away over the surface of the specimen. A variety of materials are suitable for this purpose. Non-curing modelling clay, such as plasticene, is widely available and easy to use, although dark colours may stain the surface of pale specimens, and silcone rubber may adhere to some clays (see Goodwin and Chaney 1994). For walls surrounding small areas, rapid curing two part vinyl polysiloxane rubbers designed for dental applications, such as Colténe® President, can be very effective in forming mould walls although they tend to be expensive.

As for the moulding material itself, many different RTV rubbers are available from a number of manufacturers and suppliers, and the choice of rubber will depend to some extent on the nature of the specimen and the mould required. For moulding microscopic details of fossils, however, the uncured rubber will need to be of low viscosity, and the cured rubber should have a high elongation at break and high tear strength. These attributes will maximize the chances of removing the mould cleanly from the fossil without leaving torn-off fragments stuck in the deeper recesses and undercuts (which can be very difficult to remove without causing damage). When dealing with fragile material, however, high tear strength rubbers can increase the possibility of damage to the specimen because the specimen may break before the rubber tears, so careful consideration of the properties of the cured rubber is important. Having tried a number of different rubbers, I have had good results with a product sold as Ambersil RTV913 in the UK (manufactured by Ambersil Silicones) or as QM113 in North America (Quantum Silicones, www.quantumsilicones.com). Distributors in other parts of the world are listed on the UK manufacturers website (www.amberchemical.com). This rubber is a two component room temperature condensation curing silicone compound. The cured rubber is very flexible and has good shelf-life stability. Full details of the physical properties are available from Ambersil (www.ambersilsilicones.com), but the important attributes are as follows (manufacturers data): viscosity of base compound approximately 15,000 MPa.s (MilliPascal seconds; the viscosity of the compound with catalyst added is slightly less); linear shrinkage < 0.5%, tensile strength of cured rubber, 3.1 Mpa; elongation at break, 650%; tear strength 22 kN/m. The potlife of the catalysed rubber (i.e., the working time for mould pouring) is approximately 45 minutes.

The rubber is mixed according to the manufacturers instructions, taking appropriate precautions for the safe handling of the materials (see health and safety information below). Optical imaging of the mould may be desirable, in which case black pigment (e.g. Aniline Black or Lamp Black) can be added to the rubber at the same time as the catalyst. After mixing, placing the rubber under vacuum for a few minutes can reduce the number of bubbles, but when applying rubber as outlined here I have not found this step to be necessary. All the examples presented below have used Ambersil RTV913.

The quality of the final mould depends to a large extent on the way in which the catalysed liquid rubber is applied to the specimen. The best results are achieved by trickling a small amount of rubber down the side of the wall or onto the specimen, and allowing it to flow slowly over the surface, so that the rubber creeps over the specimen, and is pulled into details and recesses by surface tension and capillary action. If the advancing edge of the rubber is allowed to bulge outwards and “roll” over the surface of the specimen there is a high probability that air bubbles will be trapped in fine details and recessed areas. Using a mounted needle or something similar to gently pull back the advancing edge of the rubber, taking care not to contact the surface of the specimen, can slow the rate of advance and prevent the edge from rolling over. The rate of flow is also much easier to control if liquid rubber is added in small increments. Cured RTV913 is very flexible and lacks rigidity. For small specimens or moulds this flexibility does not present a problem, but larger moulds may require a rigid supporting jacket (or mother mould), or addition of a layer of stiffer RTV around the mould.

The next stage of preparation will depend on the nature of the material, and whether it is the mould of the specimen that is of interest or a replica of the original. In cases where rubber is being used to cast a natural mould or a mould prepared by acid preparation of a specimen (see Example 1), it will be the rubber cast itself that is of interest. Once cured this rubber cast can be mounted and coated for scanning electron microscopy using standard methods (although because of the poor conductivity of the rubber, longer coating times may be required than for most specimens). If a replica of the original specimen is required (see Examples 2 and 3), an epoxy resin cast should be prepared.

Epoxy Resin Casting

In making high resolution casts, the choice of casting medium depends on a number of factors, but for detailed replication of microscopic details on small specimens, epoxy resins are ideal. Not only are epoxy casts rigid and durable, their fidelity to the mould is very high, better than any other material (Chaney 1989), and they are stable over long periods. They are also easily mounted and coated for scanning electron microscopy. One factor that should also be borne in mind is that not all resins and moulding materials are compatible, and reactions between the mould and the casting medium can significantly reduce the quality of the cast. For reviews of the problems relating to compatibility between epoxies and silicon-based moulding compounds in the context of reproducing tooth wear facets and details of microwear see Gordon (1984) and Teaford and Oyen (1989), or Bromage (1985) for more general comments on replicas in scanning electron microscopy. After the issue of compatibility, probably the most important factor in determining the quality of reproduction of microscopic detail is the viscosity of the resin. Low viscosity resins flow more easily into small recesses and are less likely to trap air bubbles.

I have had good results with Araldite 2020, a widely available two-component low-viscosity, water-white epoxy adhesive which is also suitable for casting (see Examples 2 and 3). Not only is the viscosity low enough for epoxy to flow easily into details of the mould, it is compatible with RTV 913, yielding good quality casts with high levels of detail (including features only a few micrometres or tens of micrometres in size [see Example 3]). According to the manufacturers technical support department, casts in Araldite, if stored away from UV light, should last at least 50 years, although projection beyond that time is difficult (Noel Moss, Vantico Ltd., personal commun., 2003).

The process of pouring an epoxy cast is similar to that for preparing a rubber mould outlined above. The epoxy resin and catalyst are mixed according to the manufacturers instructions, taking appropriate precautions for the safe handling of the materials (see health and safety information below). The usable life of the catalysed epoxy is about 45 minutes at 23°C (but note that the exothermic reaction between catalyst and resin can cause a rapid rise in temperature if large amounts of resin are mixed, and this temperature increase will accelerate curing). Small batches of epoxy weighing only a few grams can be prepared if the proportions of catalyst and resin are measured by weight using scales of appropriate precision. The catalysed epoxy is then trickled slowly into the mould, or added drop by drop, and allowed to flow slowly over the surface. The use of hand-driven centrifuges is advocated in some discussions of epoxy casting (e.g., Waters and Savage 1971), but I have not found this step necessary when pouring one-part moulds using Araldite 2020. The epoxy is water clear, so if optical examination or imaging will be required, pigment should be added to the resin at the same time the catalyst is mixed in. Addition and thorough mixing-in of Lamp Black or Aniline Black pigment produces opaque black casts. Other pigments suitable for epoxy may also be used. It is worth noting that if the epoxy is coloured using a solution of eosin stain in ethanol, the method of Jernvall and Selänne (1999) can be used to acquire high-resolution digital representations of fossil shape through laser confocal microscopy (although the effects of eosin solution on the cure properties Araldite 2020 have not been tested). For Scanning Electron Microscopy, it makes no difference whether the cast is pigmented or clear.

Health and Safety and RTV Rubber and Epoxy

Care must be taken, when using RTV rubbers and epoxy resins, to follow manufacturer instructions regarding safe handling and storage. Some of the components of RTV rubbers, epoxy resins and their catalysts are toxic and/or irritants. Material Safety Data Sheets (MSDS) for Ambersil RTV913 and other Ambersil products are available via the Ambersil and Quantum Silicones websites (www.ambersilsilicones.com, www.quantumsilicones.com). Instructions on how to obtain MSDS for Araldite 2000 series epoxy resins are available from the distributors website (www.adhesives.vantico.com).

Stereo Anaglyph Images

Techniques for obtaining and publishing stereo images of fossils, and especially small fossils, are widely known in the palaeontological community, and their greater use has been advocated by a number of authors over the years (Evitt 1949; Sylvester Bradley 1971). Conodonts were among the first fossils to which stereophotographic methods were applied (Branson and Mehl 1933; Evitt 1949), yet with the exception of ostracods (Siveter 1984) stereo images are seldom employed to illustrate microfossils, or the microscopic details of larger specimens. The standard approach is to obtain two images of the fossil which differ in their effective viewing angle by 8-10° (although 5° may give better results when the depth of a specimen, in the orientation being photographed, exceeds the average width; Sylvester Bradley 1971). Optical or digital stereo photomicrographs can be obtained using simple tilting or sliding stages (Branson and Mehl 1933; Feldman 1989); scanning electron micrograph pairs are easily obtained by tilting the specimen stage. Once obtained, the images are reproduced side by side, with the axis of image rotation aligned vertically between them, and viewed using stereo viewers (see Feldman 1989 for details). This method can be very effective in communicating three-dimensional (3D) geometries of fossils, but the size of the images is limited, and unless large format stereoscopic viewers are used, the maximum width at which images can be reproduced is only 5 or 6 centimetres (see Sylvester Bradley 1971 for discussion).

What is perhaps less well known among palaeontologists is that red-green or red-blue anaglyph images offer an alternative that with the widespread availability of powerful image editing software such as Adobe Photoshop® and Correl Photopaint® are quick and simple to produce. Images can be reproduced at any size (for printing, viewing on screen, or even for projection in presentations) and viewed in stereo with readily available and inexpensive cardboard and plastic viewers (with one red and one green lens, or one red and one blue, depending on the chosen anaglyph format; viewers are available from scientific equipment suppliers, or from the numerous potential vendors that are revealed by a www search for “3D glasses”). Stereo images produced in this way can be extremely useful as research tools, for communication via the Internet and email, and also for publication. Reproduction of colour plates and figures is now more widespread and cheaper than in the past, and is even made available at no additional cost by some journals.

Knappertsbusch (2002) recently outlined a method combining digital stereo imaging through an optical microscope with extended focus montaging and QuickTime VR™ authoring to produce animated anaglyph stereo images of microfossils. He highlighted some of the advantages of anaglyph stereo images and considered aspects of stereo image acquisition in some detail, but the paper was focussed on a particular method, using relatively expensive hardware. My goal here is to provide a simple guide to the production of anaglyph images using widely available equipment and software.

The exact method of producing anaglyph stereo images varies according to the software package used, but Appendix 2 to this paper includes step-by-step guides to producing images using Adobe Photoshop®. A set of actions that will automate much of the process in Photoshop® is also available for download. Producing anaglyph images using software packages that do not support image layers is less intuitive, but a www search for “analglyph” brings up instructions for many common digital imaging or photo editing packages that should be readily applicable to palaeontological images.

EXAMPLES OF APPLICATION OF SILICON RUBBER CASTING, EPOXY REPLICATION, AND STEREO IMAGING

Example 1: Silicon Rubber Casting of Acid Prepared Mould of a Natural Assemblage of *Distomodus*

Specimen BGS MWL 4702 preserves a partial skeleton of a conodont, but the identity of the taxon could not be determined because the P elements were exposed on a slab of black shale with their lower surface uppermost. The taxonomically diagnostic features of the elements were facing downwards, embedded in the matrix. After taking a silicon rubber mould to preserve a record of the surface of the specimen, it was prepared by carefully dissolving the phosphatic crown tissue of the elements in 10% hydrochloric acid. When dry the surface was consolidated with pvb in methanol, and a cast of the external mould of the elements made using RTV 913. After curing, the rubber cast was removed, mounted and coated (with silver) for scanning electron microscopy. Stereo pairs were prepared by acquiring images of the specimen at approximately –5 and +5 degrees relative to horizontal. Red-green anaglyph stereo images (Figure 1) were prepared by the method outline above and

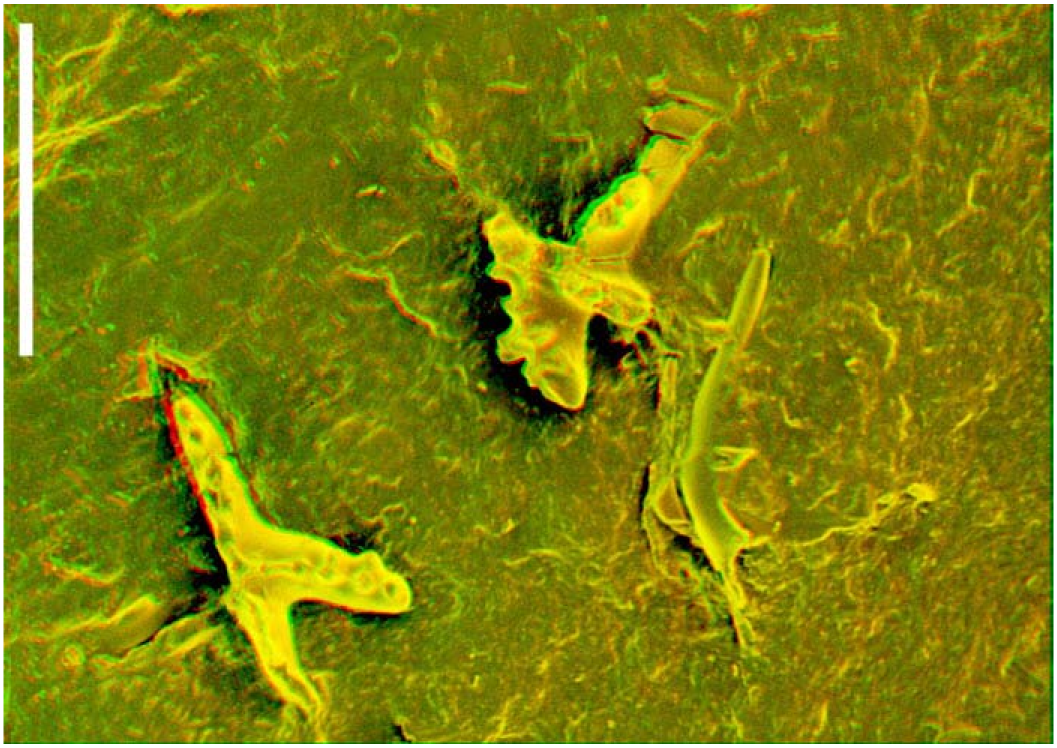

Figure 1. Red-green anaglyph stereo image of RTV 913 cast of BGS MWL 4702, a partial skeleton of the conodont *Distomodus*, showing oral surfaces of P elements. Scale bar 1 mm.

detailed in Appendix 2. These images have been used for primary research on the specimen (as the only way of visualising details of the surface of the P elements in 3D), for communication with colleagues and collaborators (email, presentations, and reports), and for publication (Purnell et al. 2003).

Example 2. Acid Preparation, Consolidation, Silicon Rubber Moulding and Epoxy Casting of the Mouth of *Protopteraspis vogti*

Specimen A28720 (Paleontologisk Museum, Oslo) was collected from the Devonian, Ben Nevis Formation during the 1925 expedition to Spitzbergen. The slab preserves articulated remains of several individuals of *Protopteraspis vogti* Kaier, including two with articulated oral plates. Heterostracans preserving articulated oral plates are rare, and the outer surface of one of the specimens on the slab, A28720/2, was mechanically prepared to expose the details of the mouth (by Anatol Heintz, sometime between 1925 and 1930). The specimen lies at an angle to the surface of the slab, and as a result of the removal of material from the mouth the exposed oral plates now sit within a steep-sided recess, more than 1 cm deep (Figure 2). As part of an investigation into the structure of the mouth of pteraspid heterostracans (Purnell 2002, and ongoing work with D. K. Elliot), detailed images of the mouth were required. The fact that the specimen sits on a recess caused some difficulties, especially in trying to obtain scanning electron photomicrographs.

Additional acid preparation of the specimen was carried out to remove carbonate rock matrix from around the oral plates of the mouth using ethanoic acid (acetic acid), buffered according to the method of Jeppsson et al. (1985). Areas of the specimen which required no further preparation were protected from the acid by a covering of pvb, and as matrix was removed by acid dissolution, newly exposed areas of the oral plates were also covered with pvb. Prior to moulding, these protective coatings were removed, and a thin layer of pvb was applied as a precautionary separator over the area to be moulded (following the procedure outlined above).

Figures 2B and 2C shows scanning electron images of an epoxy replica of the mouth of *P.vogti* specimen A28720/2. The replica was cast using Araldite 2020 in a mould of RTV913. The figure shows the level of detail revealed by the acid preparation and retained through the moulding and casting process. Because the specimen sits in a recess, the anterior view of the mouth (Figure 2C) could not have been obtained by direct optical or scanning electron microscope imaging.

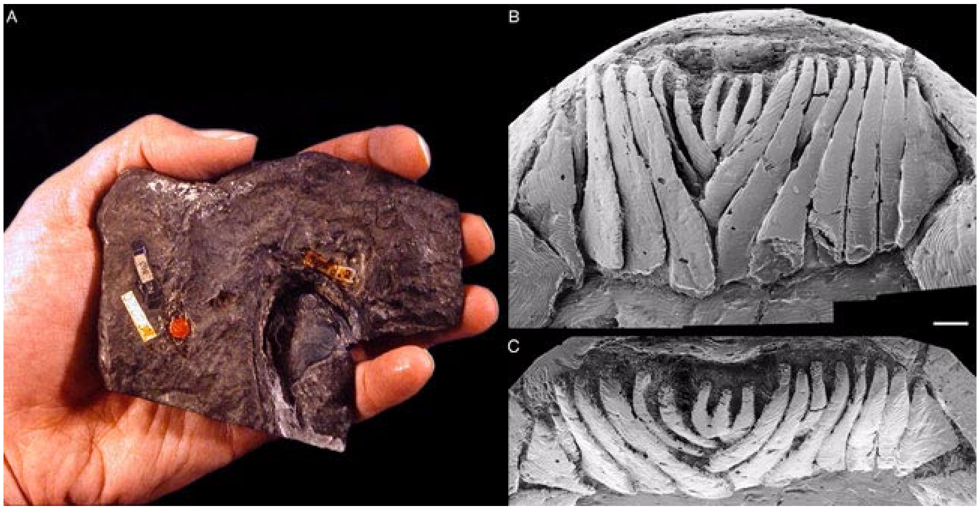

Figure 2. Specimen A28720 (Paleontologisk Museum, Oslo) preserving articulated remains of the heterostracan *Protopteraspis vogti* Kiaer (specimen A28720/2). A. Whole specimen showing the mouth of the heterostracan sitting with the recess resulting from mechanical preparation to reveal the oral plates. B. Montage of scanning electron photomicrographs of epoxy resin replica of the oral plates following acid preparation, consolidation, and silicon rubber moulding. C. Montage of scanning electron photomicrographs of epoxy resin replica, showing the oral plates in oblique anterior view. Scale bar 1 mm.

Example 3: Acid Preparation, Consolidation, Silicon Rubber Moulding and Epoxy Casting of the Apparatus of Conodont Specimen RMS GY 1992.41.2

National Museum of Scotland specimen RMS GY 1992.41.2 is one of the 10 specimens from the Granton Shrimp bed of Edinburgh that preserve traces of soft tissue (Aldridge et al. 1993). Although the exceptional preservation of body remains has transformed the study of conodont palaeobiology, studying the conodont elements in some of the specimens has been rather problematic because of the difficulties of preparing elements without damaging soft tissue remains. In some cases it has not been possible to assign a specimen to a taxon, simply because the identity of the elements could not be determined due to significant morphological details being obscured by rock. Also, scanning electron microscope imaging of elements in some of the specimens has been prevented because the blocks are too large.

Interpretation of specimen RMS GY 1992.41.2 has been hampered by both these problems. Aldridge et al. (1993 p. 420) noted that in this specimen "The Pa element is not sufficiently exposed to allow the taxon to be positively identified." The conodont lies in the centre of a large block of laminated, organic rich limestone (16-20 cm long, 9-12 cm wide, 8-9 cm thick), thus effectively preventing imaging of the elements in a scanning electron microscope.

In order to overcome these difficulties, acid preparation of the P_1 element (= Pa, see Purnell et al. 2000 for discussion of element notation) was undertaken, using pvb to coat adjacent areas and limit the effects of the acid to the area of the P_1 element only. Buffered nethanoic acid (formic acid), prepared according to the method of Jeppsson and Anehus (1995), was applied to the element and its immediate surroundings drop by drop. The reaction was constantly monitored under a binocular microscope to ensure that the specimen was not adversely affected in any way. The area under preparation was washed periodically with deionised water, and a fine paintbrush (00) and a very fine flexible steel mounted needle (0.25 mm diameter) were used to assist with the removal of the matrix from around the element. After several hours, the element was considered sufficiently well exposed and, after washing, drying, and application of a thin layer of pvb as a separator, a rubber mould was made using RTV 913 contained with a wall of Colténe® President following the method detailed above. From this step an epoxy cast was made using Araldite 2020 (see above).

Figure 3A shows an anaglyph stereo image of the epoxy replica of the elements preserved in specimen RMS GY 1992.41.2. The P_1 element, toward the left is now well exposed. The enlargements show the level of detail reproduced by the replica, with incremental growth lamellae evident in the basal cavity of the P_1 (arrowed in Figure 3B)

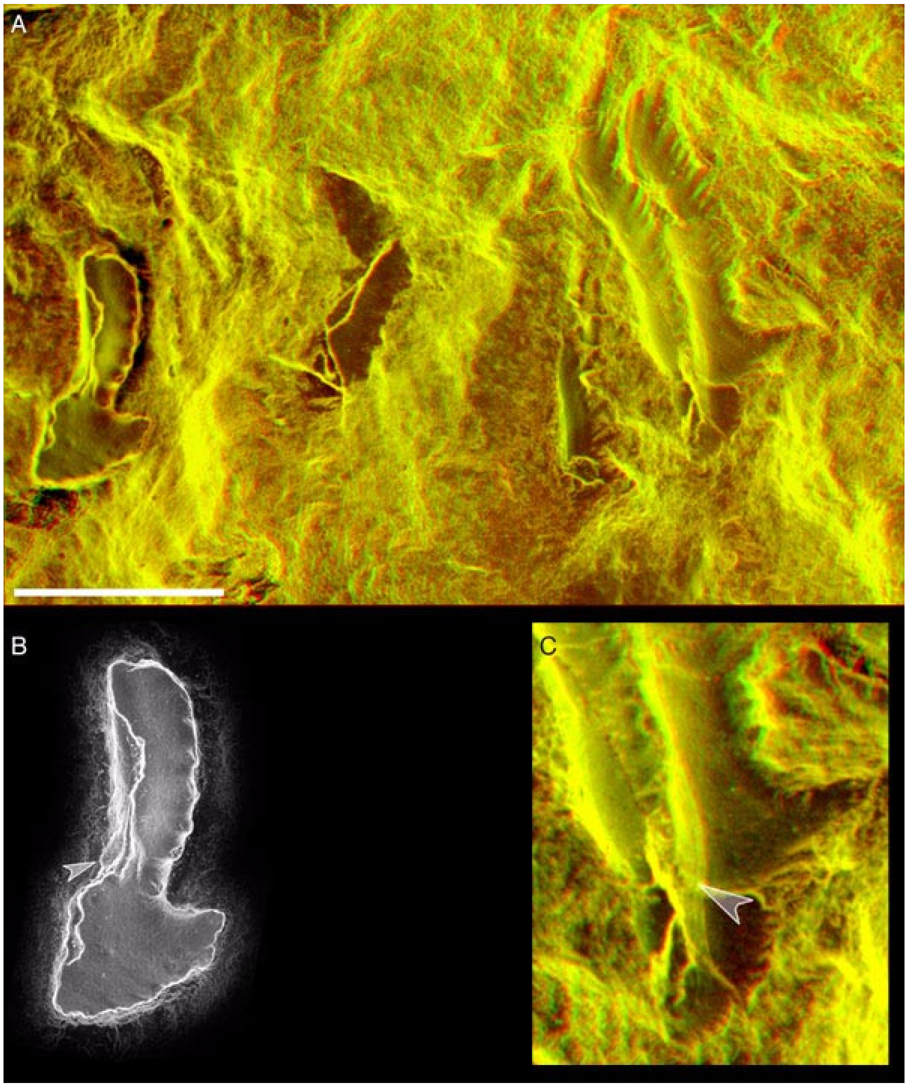

Figure 3. Epoxy replica of the apparatus preserved in conodont specimen RMS GY 1992.41.2 (Royal Museum of Scotland). A Red-green anaglyph stereo image of whole apparatus after acid preparation of the P_1 element (at left). B. Close-up of the epoxy replica of the P1 element showing incremental growth lamellae of the crown tissue exposed in the basal cavity (arrowed). C. Red-green anaglyph stereo image close-up of the S3 and S4 elements, showing incremental growth lamellae of the crown tissue exposed along the lower margin of the S4 element (arrowed). Scale bar 0.5 mm.

and along the lower margin of the S4 elements (arrowed in Figure 3C).

CONCLUSIONS

Silicon rubber moulding, epoxy casting, and anaglyph imaging provide effective, relatively simple and inexpensive techniques for the replication and illustration of microscopic details of fossils, details that for a variety of reasons may not be amenable to study using other methods. The techniques presented here indicate how to maximise the level of detail that can be replicated and imaged with minimum risk to the fossil. With the widespread use of digital imaging, combined with electronic communication and publication, anaglyph images offer a simple, inexpensive and extremely effective means of illustrating fossils in three-dimensions.

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APPENDIX 1

A step-by-step guide to producing red-green anaglyph stereo images from greyscale images using Adobe Photoshop® (tested with versions 4 upwards). To view the final image in stereo, red-green viewers (3D glasses) are required. If red-blue viewers are to be used, follow the instructions below, but at step 5 set the output levels of the red and green channels (not red and blue) to 0.

1. Open the files for the left and right images of the stereo pair and ensure that they are correctly oriented, with the axis of rotation (8-10°, unless vertical exaggeration is desired) aligned vertically. The process is easier if the images are of the same pixel dimensions, and it is usually helpful to give the files names that indicate which image is left and which right (in this example they are called Xleft.tif and Xright.tif).
2. Taking one image first, in this case Xleft.tif, the image for the left eye (acquired at – 4° relative to horizontal), from the **Image** menu, select **Mode > RGB Color**.
3. From the **Image** menu, select **Adjust > Levels** (or **Adjustments > Levels** in Photoshop 7).
4. a. In the **Levels** window, from the **Channel** pop-up menu, select **Green**.
   b. Set the **Output Levels** to 0 and 0. The image should now appear purple.
   c. Stay in the **Levels** window, and from the **Channel** menu select **Blue**.
   d. Set the **Output Levels** to 0 and 0. The image should now appear red.
   e. Stay in the **Levels** window, and from the **Channel** menu select **Red**. If the Red channel's histogram is truncated or skewed, adjust the sliders accordingly to improve image contrast and brightness (this step is optional, but can significantly improve the quality of the final stereo image).
   f. Click **OK** to close the **Levels** window.
5. Make the right image of the stereo pair (Xright.tif in this example) the active window (acquired at + 4° relative to horizontal), from the **Image** menu, select **Mode > RGB Color**.
6. From the **Image** menu, select **Adjust > Levels** (or **Adjustments > Levels** in Photoshop 7). Repeat steps 4a-b for the **Red** and **Blue** channels. The image should now appear Green. Repeat step 4e for the **Green** channel (optional).
7. From the **Layer** menu, select **Duplicate Layer**. In the **Duplicate Layer** window, in the **Destination**, **Document** pop-up menu, select the file containing the left image (in this case Xleft.tif) and click OK.
8. Make the left image the active window (in this case Xleft.tif). It will now have two levels (shown in the **Layers** palette) the uppermost of which will be the right image that you just duplicated in step 7.
9. In the **Layers** palette, from the **Layers** pop-up menu select **Screen**.
10. If necessary, adjust the position of the uppermost layer to maximise the alignment of the two images. The anaglyph stereo pair is now complete and should look three-dimensional when viewed using red-green viewers. The image should be saved in the format best suited to the purpose of the image. To avoid loss of information through image compression and preserve the layers, which will allow for future adjustments to be made, save as a Photoshop format file. If the image is being prepared for publication, a file format that does not involve loss of information during compression is best, probably TIFF or EPS (**not** JPEG). For use on the www or in a Powerpoint presentation, adjust the pixel dimensions of the image downwards to a size that is suitable (i.e., no more than 1024 x 768 for Powerpoint), and if no further editing will be required, save as a JPEG file, with appropriate compression.

A Photoshop actions file (Anaglyph_RG.atn for Photoshop® v. 4 or newer) is available for download. When loaded into Photoshop®, the two actions (LeftToRed and RightToGreen) will automate steps 2-4 for the left image and and steps 5-6 for the right image. (To load these actions, choose **Load Actions** from the **Actions** palette menu. Locate and select the action set file [Anaglyph_RG.atn], then click **Load**). For more details regarding actions consult Photoshop Help or the Manual.

APPENDIX 2

Product information, manufacturers, suppliers, and distributors

www.amberchemical.com: Ambersil Silicones, UK manufacturer of RTV 913 and other rubbers; website also lists distributors for other parts of the world, and has request forms for Material Safety Data Sheets.

www.quantumsilicones.com: Quantum Silicones, US supplier of RTV 913 (as QM113).

www.adhesives.vantico.com: distributor for Araldite 2000 series epoxy resins; website also includes instructions on how to obtain MSDS

www.spnhc.org: The Society for the Preservation of Natural History Collections, including links (under “publications”) to SPNHC Leaflet #2 Adhesives and Consolidants in Geological and Paleontological Applications (Elder et al. 1997).