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Title: Resin-embedded anatomical cross-sections as a teaching adjunct for medical curricula: Is this technique an alternative to potting and plastination?

Short title: Resin-embedded cross-sections for anatomy teaching

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Abstract

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With an ever expanding use of cross-sectional imaging for diagnostic and therapeutic purposes, there has also been an increase in the need for exposure to such radiological and anatomical views at the undergraduate and postgraduate level to allow for early familiarisation with the relevant anatomy. Cadaveric cross-sections offer an excellent link between the two-dimensional radiological images and the three-dimensional anatomical structures. For such cross-sections to be useful and informative within educational settings, they need to be: i) safe for students and trainees to handle; ii) robust enough to withstand repeated handling; and iii) display anatomy clearly and accurately. There are various ways in which cross-sections can be prepared and presented; plastinated, potted, vacuum sealed or unmounted. Each of these approaches have advantages and disadvantages in terms of technical complexity, cost and quality. As an alternative to the above methods and their limitations, we propose the presentation of cadaveric cross-sections in a transparent polyester resin. This technique has been used extensively in craft and artistic industries, yet it is not publicised in anatomy teaching settings. The sections are layered in polyester resin contained within a mould. The set resin required finishing by sanding and polishing. The final cross-sections were safe to handle, durable and maintained excellent anatomical relationships of the contained structures. The transparency of the set resin was water-clear and did not obstruct the visibility of the anatomy. The cost of the process was found to be significantly lower, requiring less infrastructure when compared to alternative methods. The following trivial technical difficulties were noted during the resin-embedding process: trapped air causing organs to float; retained water in the anatomical specimens creating bubbles and discolouration; and microbubbles emerging from the solution affecting the finished surface. However, solutions to these minor limitations have been discussed within the paper with the aim of future proofing this technique. The sections have been used in undergraduate medical teaching for four years and they have shown no signs of degradation or discolouration. We believe that this method is a viable and cost effective alternative to other approaches of displaying cross-sectional cadaveric material and will help students and trainees bridge the gap between the traditional three-dimensional anatomy and two-dimensional images.

Introduction

Healthcare professionals employ a range of different imaging techniques either to aid the diagnostic process or even as part of therapeutic regimes for patients. In such instances, the normal structure and any pathologies that may be present are assessed in different anatomical planes to allow for an accurate decision or inform a management plan accordingly. However, without a solid foundation of anatomical knowledge, some of these views can be challenging when it comes to their interpretation. This has resulted in an increase in the call for cross-sectional anatomy teaching to help augment students and trainees' understanding of the anatomy displayed in modern imaging techniques. The aim is to introduce challenging planes, especially axial views, early in undergraduate medical curricula and integrate cadaveric anatomy with future radiological images that would ultimate enhance future clinicians' skills. (Chowdhury et al., 2008. De Barros et al., 2000. Miles, 2005.)

One way to accomplish an integration between normal structure and radiological interpretation is to have cadaveric cross-sections to help bridge the gap between three-dimensional anatomy and the two-dimensional images. The choice of how to display these sections has to take several factors into account. Anatomical cross-sections have to be displayed in form that is: i) safe for students and trainees to handle; ii) robust enough to withstand repeated handling; and iii) display anatomy clearly and accurately. In our experience there are several different methods currently in use to meet this need such as mounted specimens in an acrylic pot, plastination, vacuum sealed specimens and un-mounted specimens in a box or tray. Drawing from personal experiences and anecdotal evidence amongst the anatomy community, each of these methods has its advantages and disadvantages as listed in table 1.

The question asked is whether there is an alternative way to embed anatomical cross-sections in a substrate that would allow them to be readily handled whilst being cost effective and safe for students and trainees. We conducted several discussions with an experienced model maker that revealed the process of clear casting in polyester resin. This technique is well used in craft and artistic industries, yet not publicised in anatomy teaching settings. Further investigation into the available literature highlighted that this technique in relation to anatomy is actually not a new idea with Tompsett (1957) being amongst the first authors discussing its use and more recently with Oliveira et al (2013) presenting positive results for slices and whole organs embedded in resin. Grimsrud and Dugstad (1975) mention its ubiquitous use but unsuitability for use in brain sections. As mentioned earlier, this process in its self is well documented in the craft's sector and many of the sources of information came from that industry. (Resin-supplies.co.uk. (2017). Castin' [sic] Craft Casting Resin basics, Instructions and tips, Eti-usa.com, n.d.)

The main purpose of this paper is to provide a comprehensive and reproducible description of the methodological steps involved in the process of developing resin-embedded transverse cross-sections that can be subsequently employed as a teaching adjunct for anatomy at the undergraduate and postgraduate level. Our aim was to present the cross-sections following the standard axial radiological convention, used for Computed Tomography (CT) and Magnetic Resonance Imaging (MRI), according to which healthcare professionals and trainees look at a supine patient from the feet up (i.e. patient's left side is on the right side of the radiological image) and therefore the quality of the anatomical inferior side of each specimen was of paramount importance. The preparation, embedding, and finishing steps are discussed in detail including important learning points to ensure future proofing of this technique.

Methods

The process of developing resin-embedded cross-sections in the transverse plane encompasses three main stages: i) an initial step that entails a methodical preparation of the anatomical specimens; ii) a subsequent phase of embedding these anatomical specimens into the chosen medium and iii) the finishing step during which the cross-sections are checked for quality assurance to ensure a high standard for undergraduate and postgraduate anatomy teaching. These steps and their technical requirements are described in the following sections.

Ethical Considerations

A suitable donor was identified from the University of St Andrews bequest programme with written permission, granted by the donor at the time of registering and as documented at the bequest declaration form, to retain parts of the body for further education and training purposes. The selection of the donor and the following steps for resin embedding of the cross-sections were performed in accordance with the Anatomy Act (1984) and the Human Tissue Act (Scotland) 2006 under the auspices of the senior licensed teacher of anatomy from the University of St Andrews, UK.

Preparation Step

The selected cadaver was embalmed via the femoral artery, to avoid disturbing neck anatomy, with Vickers Cambridge Mix© fluid. This is predominantly a formaldehyde-based solution that has been widely used to preserve cadavers for anatomical examination in the UK. The exact contents of the Vickers Cambridge Mix© are listed in table 2.

Three months after embalming, the cadaver was removed from storage and the limbs were separated at the level of the upper arm and upper thigh. The cadaver was then placed in a freezer at -20°C for 48hrs. After this time period, the cadaver was removed from the freezer and the following anatomical planes of most interest, (fig 1) were marked: sternal angle (joint between the manubrium and sternal body – approximate level of T4/T5), transpyloric plane (halfway between the jugular notch and the pubic symphysis – approximate level of L1), transtubercular plane (at the level of the iliac tubercles – approximate level of L5). Using an AEW 400 bandsaw, transverse sections were cut starting from rostral and progressively moving to caudal regions of the body. The goal, while undertaking this step, was to land to the aforementioned anatomical planes when making the cuts and also to complete the sectioning as swiftly as possible without allowing for tissue thawing. Sections were cut between 1cm and 2cm in depth. The head sections were specifically cut in parallel to the orbitomeatal line (a line from the outer canthus of the eye to the centre of the external auditory meatus). This plane was chosen to match our collection of in-house CT images and because of the ease of determining the surface landmarks in a fixed and frozen cadaver. Inferior to the head, sections were cut following a true anatomical transverse plane.

After completion of sectioning, the transverse sections were positioned with the anatomically inferior side facing superiorly on top of trolleys lined with absorbent paper, allowing them to thaw and dry. All sections were allowed to air dry in licensed premises, which are temperature controlled at approximately 16.5°C with low levels of humidity, for a period of between 5 and 8 days.

Embedding Step

The chosen medium was a pre-accelerated, unsaturated polyester resin in styrene monomer, commercially available as ‘clear casting resin’. When a Methyl Ethyl Ketone Peroxide (MEKP) catalyst is added, this medium rapidly hardens while becoming clear. The polymerisation reaction is highly exothermic and produces noxious fumes therefore the setting process took place in well-ventilated licensed premises. The transverse cross-sections were embedded in stages as described below.

The resin was mixed in small batches, of 300g at a time, in large disposable containers. We ensured that all working benches and surfaces were covered in heavy-duty plastic or similar material and only disposable equipment was used, as resin creates a hard almost permanent coating on anything that it comes in contact with, significantly limiting its future usability. Using a syringe, 1% by mass of MEKP catalyst was slowly added, to prevent inclusion of air, into resin. The mixture was then stirred, using either a plastic or metal stirrer slowly. The stirring was continued until the mixture was of even appearance with strands of polymerised resin beginning to appear. At this point the resin had a light green colour, which disappeared gradually as the polymerisation process started taking place, leaving a transparent medium. As resin begins to set almost immediately after the addition of the MEKP catalyst, the mixture was decanted as soon as possible into suitable moulds to a depth of 5mm to 10mm to form the base of each cross-section. For our moulds, this equated to approximately 1kg of resin mixture. At this point any visible air bubbles were removed by either piercing them with a probe or moving them to the edge of the mould and then compressing them against the wall of the mould. We opted to use polypropylene storage boxes of appropriate sizes depending on the body region being embedded (e.g. 35cm x 26cm for head and abdomen, 51cm x 32cm for thorax and pelvis). A variety of different mould materials are suitable for resin embedding with examples including metal and glass (Tompsett, 1957). However, acrylic should be avoided as it will bind together with the resin requiring manual separation of these two substances that can adversely hamper the embedding process and hence the overall quality of the cross-sections.

The base of each cross-section was allowed to set until it reached a gel-like state capable of supporting the combined weight of the intended cross-section. With the size of our chosen moulds, this step required approximately 90min. Each cross-section was then carefully placed into the mould on top of the base layer with the anatomically inferior surface facing upwards. Care was taken to maintain the anatomy in place during the transfer to the mould. For the transfer and placement into the moulds, we placed a sheet of ridged plastic under each anatomical slice to aid this process. At this stage, an identification tag with the body ID number and the individual number of the slice was also placed alongside of the anatomical specimens per resin cast. This allows for the cross-section to be identifiable, in accordance with the anatomy legislation in Scotland, UK (Legislation.gov.uk, 2017)

Once the anatomical specimens were placed in each corresponding mould, more mixed resin was poured over the top and around each section. The two layers of resin bound together leaving an imperceptible joining line that does not affect the quality of the finished cross-sections. We continued adding resin until each anatomical specimen was submerged by approximately 5mm. It is important to note that the amount of resin required will vary depending on the size of the mould and the thickness of the anatomical sections. At this stage, a probe was used in an attempt to free any air trapped underneath the tissues. Structures were lifted and resin allowed to flow underneath. We continued observing the anatomical specimens and resin casts for any further escape of bubbles. As these emerged they were moved to the side of the mould, using a probe, and popped. For the embedding process to be successful, yielding cross-sections of high quality, air bubbles should be dealt promptly before the resin sets permanently.

The time required for the second and final layer of resin to set varies dependant on the volume of resin used. In our case, this layer required a full day to ensure a solid set before removing the cross-sections from each mould. The areas of the finished cross-section that have not been in contact with air (i.e. sides and bottom) will be hard, smooth, and very transparent. The upper surfaces, which have been in contact with the air, will have a sticky feel and a slightly opaque appearance requiring a finishing step.

Finishing Step

Once removed from the moulds, cross-sections were allowed to further set for at least a week before any additional work was carried out. This phase enabled the completion of the polymerisation reaction along with a reduction of the tackiness of the upper surface. During this time, cross-sections were inspected for soft spots. These are caused by the incomplete mixing of resin and the MEKP catalyst. Soft spots were treated with a small amount of MEKP catalyst and left to harden over time. Any larger defects caused by air bubbles were also filled with a resin and MEKP catalyst mixture carefully delivered by a syringe.

Once all surface defects were treated or filled and the surface was allowed to set, the cross-sections required additional polishing to remove the tackiness and increase the transparency. During this finishing step, if the polishing action is too vigorous it can cause the resin to melt and hence damage the equipment being used. For this reason, electric sanders are not recommended for this task. Sanding was carried out using “wet and dry” sandpaper with the surface of the section covered in water and regularly washed off. The ultimate aim was to remove as much of the surface stickiness and unevenness as possible. This can be accomplished by using a straight metal edge to scrape the surface or by making several passes with course sandpaper (100 grit or lower) under running water. This approach will also remove the sharp edge around the top.

Once the surface was even and no longer sticky and with a smooth edge, further sanding was employed. We began with 200 grit sandpaper wrapped around a cork sanding block. Using circular motions, the whole of the surface and over the edge of the top surface were sanded. We regularly washed off the swarf, which would otherwise be ground back into the surface, and wiped clean with a damp microfiber cloth. After 20min of sanding, the surface was washed and finally dried with a microfiber cloth. At this point, the surface was inspected for any areas that are not of uniform appearance. If the surface was uniform, we continued the process taking turns to increase the grit count. We used 200, 400, 800, 1000, 1500, and 2000 grit papers in order. Each stage took less time than the previous one. Once sanding with the highest grit has been completed the appearance of the upper surface will be glass smooth with a frosted tinge.

During the sanding, sub-surface air bubbles may be revealed. These may disappear as sanding continues to cut deeper into the surface. If these are significant and persistent while sanding, they should be filled with resin after polishing. If these are small, which is more likely, they can be left without any further treatment as they will not adversely affect the visual quality of the finished piece.

The next and final step is to polish each cross-section; any recognised polishing compound can be used. We tried Toothpaste, T-cut®, Brasso® and generic silver polish that all produced similar results. An electric car-polisher was used before a final hand-polish using a microfiber cloth.

Results and Discussion

Figures 2 to 5 show the finished cross-sections at the following approximate anatomical planes: sternal angle (fig 2), transpyloric plane (fig 3), and transtubercular plane (fig 4). The cross sections show the relevant anatomy clearly and accurately. Tissue has also been preserved extremely well without any major artefacts or any other issues that could have affected the quality of these sections. The clarity can be seen in close up views of a section at the sternal angle showing the carina (fig 5) and transpyloric plane showing the left kidney (fig 6).

Our stated requirements were for a display modality that should be: i) safe; ii) robust; and iii) display anatomy clearly and accurately. The resultant sections, seen above, and their use in teaching have shown these criteria to be fulfilled. The finished resin is not harmful to handle and the prototype anatomical specimen has been embedded in resin for the past four years and no tissue deterioration, degradation or discolouration has been noted. During this time sections have been used in teaching undergraduate and postgraduates and the cross-sections have maintained clear and precise anatomical fidelity without sustaining any damage. Overall, the finished resin-embedded cross-sections were exceptional in terms of long-term tissue preservation and showcasing the relevant axial anatomy, which tends to be a rather challenging view for identification of normal structures or even pathologies both at the undergraduate and postgraduate medical level. The only minor artefacts affecting the quality of the sections, noted during the development process, related to trapped air bubbles within the resin cast but remedial steps were identified and employed for the resolution of such issues as discussed in this paper.

In our introduction we compared the advantages and disadvantages of various techniques (Table 1). The main disadvantages mentioned were cost, expertise and disturbance of the anatomy. The cost to produce these cross-sections, including infrastructure, resources, equipment and materials was considerably less when compared to techniques such as potting and plastination. The calculated cost of materials per section is approximately £25 compared to a recent quote for an acrylic pot of £45 before the cost of fluid. Neither does it require the specialised equipment (vacuum chamber, acetone baths) or intensive labour of plastination (Riederer, 2013). This technique does require an involved and extensive method, however this was our first attempt at resin embedding and with little more than a brief conversation with a model maker and an few internet searches we were able to produce very positive results. In these sections structures are fixed in position and, as long as they are handled carefully during preparation, they will maintain their correct relationships indefinitely.

During the development of the method we did encounter and overcome a few problems. These problems were minor methodological considerations. One slice was lost in the transitional area between the head slices that were sectioned following the obitomeatal plane and remaining slices from neck below that were cut in true anatomical transverse planes. This resulted in a wedge-shaped anatomical slice that did not maintain its structural integrity following thawing during the preparation step. The obitomeatal plane has also more recently fallen out of favour from clinical radiology due to the unnecessary exposure of the visual lens to ionising radiation during relevant imaging investigations and instead automated reconstruction or the AP-PC line

(Anterior Commissure-Posterior Commissure) are used commonly employed nowadays in neuroimaging settings as an axial reference plane. As both of the above steps would be impractical or impossible to use within a cadaveric context, a recommended solution would be to cut all the anatomical slices, from rostral to caudal, using true anatomical transverse planes.

Floating organs, such as the brain and lungs, were also challenging to embed as they required repeated layering to cover these resulting in sections that were slightly thicker and hence heavier (fig 7). This issue was overcome by applying adhesive to the underside of the organs, which was imperceptible in the final cross-section. Surface imperfections resulting from air bubbles emerging from convoluted sections of gut needed remedial action in the form of another layer of resin being poured on top as well.

The importance of properly drying specimens was highlighted when moisture trapped in the diploic bone of the skull evaporated during the polymerisation reaction. This resulted in a white stating and bubbles around the outer edge of the skull (fig 8) and soft spots in the same areas; these artefacts were only noted in two sections. The soft spots were treated with MEKP catalyst and eventually hardened. These were the sections that had been fixed and washed after cutting, hence having retained moisture. Fortunately the discolouration did not impede on the view of the anatomy and this was merely a minor aesthetic problem.

The highly exothermic polymerisation of the resin produces noxious fumes and heat, so the process should be carried out in a well ventilated area or, if possible, a fume hood. Manufacturer's instructions state that the ratio of catalyst should be 2-3%. We used 1% to try and reduce the energy produced in the reaction.

When removed from the mould the upper surface of the section will remain tacky until the finishing steps have been carried out. Due to this, sections should never be stacked on top of one another otherwise a bonding reaction will take place between the layers in a similar way to that which occurs between the poured layers of resin in the mould. This could result in irreversible damage.

A purpose-built shelving rack (fig 9) has also been created, within the licensed premises, where the cross-sections are catalogued in order. This allows for easy, quick and accurate identification of a relevant cross-section at a desired level (i.e. T4 and L3). The cross-sections have been actively and heavily used during dissecting practical and self-directed learning to help undergraduate medical students enhance their knowledge of axial anatomy. Specifically, the cross-sections are currently being used alongside corresponding CT and MRI images with the aim of teaching integrated cadaveric and radiologic anatomy.

In conclusion, the resin-embedded cross-sections have showed excellent anatomical fidelity and tissue preservation over time. The preparation, embedding and finishing steps did not expose any major shortfalls in this technique that could be employed as a more cost-effective and perhaps easier to reproduce method when compared to potting and plastination. The on-going use of the resin-embedded cross-sections, as part of the undergraduate medical curriculum, also suggests that these are a useful teaching adjunct to augment students' knowledge of anatomy especially in relation to the axial plane.

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List of Figures

Figure 1: Planes of Anatomical interest. An outline figure showing the planes at which we made sure to place our cuts. These planes were selected by our anatomists as having the most relevance. SA – Sternal angle, TP – Transpyloric Plane and TT – Transtubercular plane

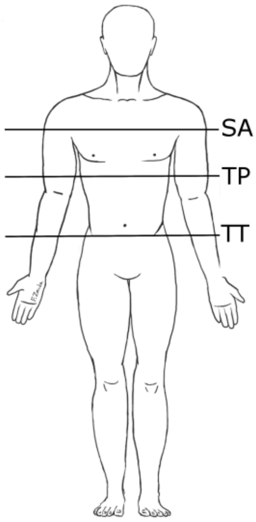

Figure 1

Figure 2: Resin embedded Transverse Section at the level of the Sternal Angle. A finished section showing the clarity of the embedding that allows for clear visualisation of the structures of the thorax. The large white mass to the left of the mediastinum is a tumour in the hilum of the right lung

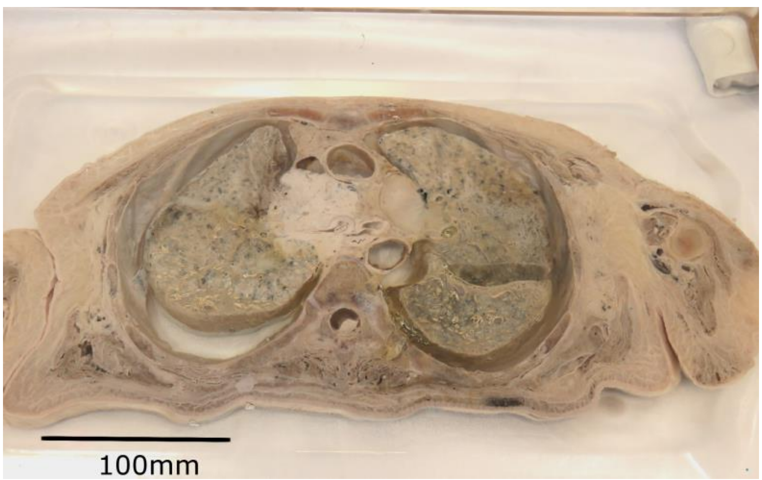

Figure 2

Figure 3: Resin embedded Transverse Section at the level of the Transpyloric plane. A finished section showing the anatomy of the upper abdomen. Clearly seen are the Liver and gall bladder on the left side of the image and the pancreas extending to the right of the image. Both kidneys are visible although the right Kidney contains a large cyst. The unusual shape of the embedding is caused by the shape of the mould used to cast the resin. Pixilation has been added to obscure the Donor Identifier.

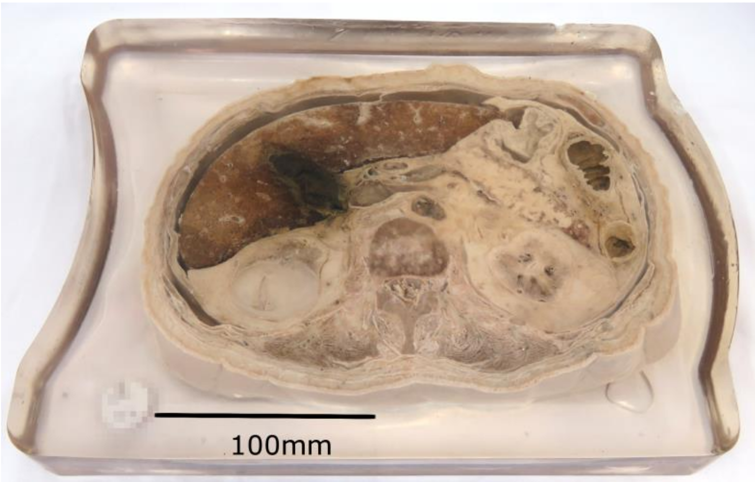

Figure 3

Figure 4: Resin embedded Transverse Section at the level of the Transtubercular plane. A finished section showing the anatomy of the lower abdomen at the level of the Iliac Tubercles. The section is not exactly transverse, slight asymmetry can be seen Iliac bones. Anteriorly, a dependant Transverse Colon can be seen crossing immediately deep to the anterior abdominal wall with the Descending Colon on the right of the image and the ascending (perhaps caecum) on the left.

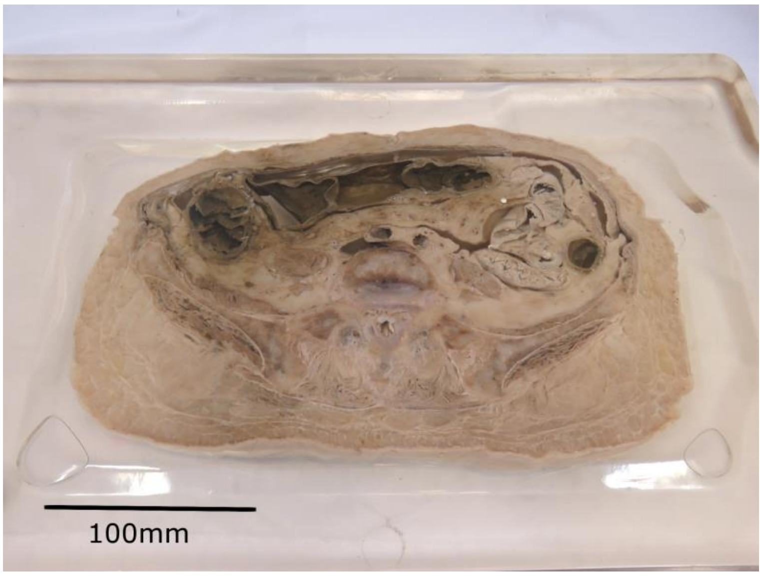

Figure 4

Figure 5: Detail View of a resin embedded Transverse Section at the level of the Sternal Angle. Structures of the mediastinum can be clearly seen in the resin even in this close up view. The labelled structures clearly identifiable even though a right side lung tumour, surrounding the Right Main Bronchus has obliterated some of the expected anatomy. RMB - Right Main Bronchus, LMB - Left Main Bronchus, O – Oesophagus, DTA - Descending Thoracic Aorta, T5 – Body of the fifth Thoracic Vertebra.

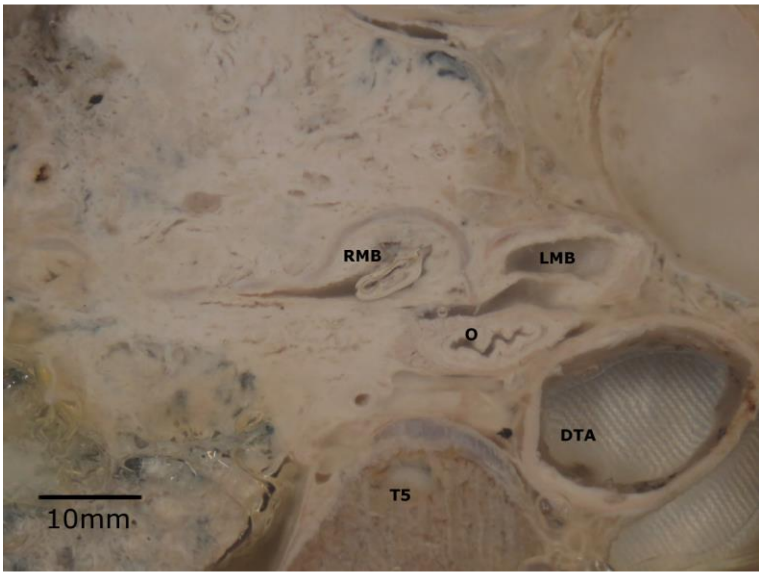

Figure 5

Figure 6: Detail View of a resin embedded Transverse Section at the level of the Transpyloric plane. A close up view of the left Kidney showing the excellent clarity of the finished

section. Clearly seen are Renal Cortex and Medulla and the surrounding Perirenal fat.

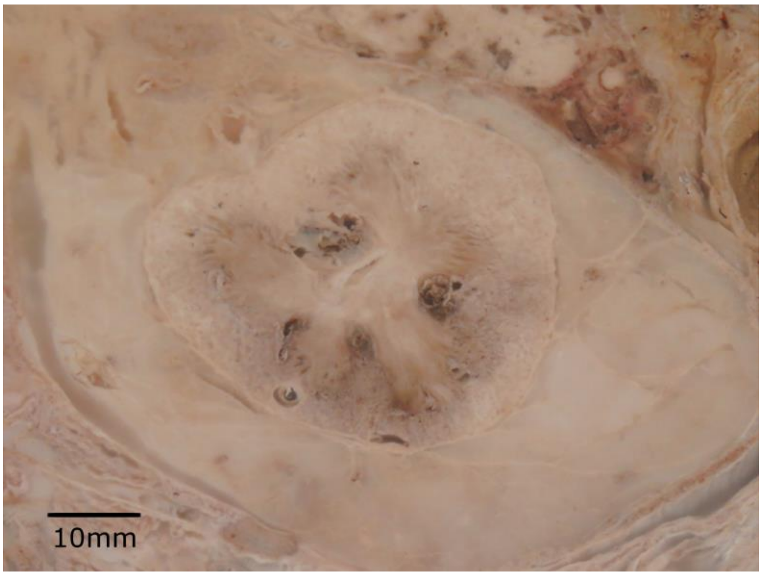

Figure 6

Figure 7: Finished sections in a purpose built rack. In order to safely and clearly display the finished sections a purpose built rack was created in the dissection room and available to students at any time. Locating a desired section is aided by the labelling of the rack and sections.

Figure 7

Figure 8: Detail view of a resin embedded transverse section of head. This close up view shows bubbles that have formed in the resin and the resultant loss of clarity. This occurs when moisture, trapped in the diploic bone of the skull, boils during the exothermic reaction of the setting resin. This bubbling and loss of clarity can be avoided by properly drying sections before embedding, although the diploic bone tends to retain moisture.

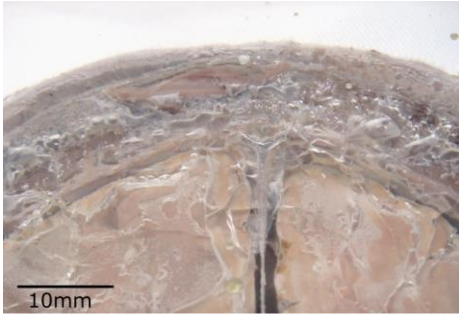

Figure 8

Figure 9: Finished sections of different depths. The section on the left is a typical depth section from the head, the section on the right is unusually thick due to the visibly displaced lung. Low density structures, such as Brain or Lung, can float in the freshly poured resin. The extra resin required to then cover the floating structures resulted in some sections being excessively thick.

Figure 9

List of Tables:

Method	Advantages	Disadvantages
Acrylic pot	Tried and tested Good longevity Structures maintained in place	Materials expensive to buy Expertise require to make Heavy and fragile
Plastination	Good longevity Ability to visualise into and around the preserved structures	Expensive infrastructure Expertise required to make Loose parts fall out
Vacuum sealed	Cheap and easy to make	Can disturb anatomy Seal can fail
Un-mounted	Low cost	Anatomy easily disturbed Prone to drying out and deteriorating

Table 1: Advantages and disadvantages of various methods of displaying cross-sectional anatomy. The table reflects the authors' experiences using the included methods for the purpose of displaying cross sectional anatomy for use in education

Content	Percentage
Ethanol	52.3%
Glycerol	24%
Water	10%
Phenol	8%
Formaldehyde	3%
Methanol	2.7%

Table 2: Vickers Cambridge Mix© Contents.