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PREPARATION OF FOSSIL BONE FOR HISTOLOGICAL EXAMINATION

by

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ABSTRACT

A technique for preparing thin sections of fossil bone suitable for histological examination by light microscopy is described.

INTRODUCTION

Palaeo-histology is the branch of palaeontology concerned with the microscopic structure of fossil bone. Researchers entering the field for the first time become aware of a need for a concise description of a technique to prepare thin sections from fossil bone. This note aims to fill that need by describing the procedure used in a recent palaeohistological study (Chinsamy 1988, 1990, 1991, 1992).

The technique described has been successfully applied to the bones of dinosaurs and mammal-like reptiles, as well as to archaeological samples of human bone and also to defatted bone of recent taxa. There is no one 'correct' method of making sections of hard tissues like bones, but all existing techniques share a number of core processes in common (Enlow 1954; Enlow and Brown 1956; Honjo and Fischer 1965; Peabody 1961; Macfall and Wollin 1972; Buffrènil, Ricqlès, Ray and Domning 1990). Although the method described here is specifically intended for use on bone, thin sections of fossilised wood have also been obtained using the same method.

MATERIALS

Many different materials and items of equipment can be used to produce perfectly acceptable results in the sectioning of bones. We list here only those materials used in the processes employed in our laboratory, as a starting point for those who might be entering the field for the first time:

Epofix cold mounting medium; Epotek; Buehler Petrographic slides (27 X 46 mm); for manual grinding, a glass plate (25 X 30 mm) and carbide powder of varying grit size (220, 400, 600, 800, 1000) are needed; for mechanical grinding, a lap wheel grinder and polisher (such as the Buehler Ecomet lll) and waterproof adhesive grinding papers (180, 220, 400, 600, 800, 1000 grit) are required; a section cutting machine such as the "Micro-trim" or any other cutting device with a diamond cut-off wheel and a resectioning vise is suitable.

METHODS EMPLOYED

The procedures we use for preparing sections of fossil bone can be conveniently divided into seven main phases:

1. Measurement and recording of data.
2. Photography.
3. Embedding of specimens for sectioning.
4. Sectioning embedded bone.
5. Grinding one surface to affix to slide.
6. Mounting and labeling the stub.
7. Finishing.

Measurement and recording of data

Because section making is a destructive process, some aspects of the gross morphology and anatomy of the bones will inevitably be lost during the process. It is therefore highly advisable, indeed essential, to record relevant data before the process begins - for example, bone size and shape are often important indicators of age, stature, sex, etc., so data relevant to these factors should be recorded before they are lost irrecoverably once sections have been cut. Making a cast of the original specimen can also provide a valuable store of information.

In order to record precisely where on the bone the section was cut, and in what orientation, and to ensure repeatability and comparability later, it is advisable to standardise any measurements taken (Figure 1). The standards should be set with reference to easily identifiable homologous points on the bone (e.g. trochanters, nutritive foramina, condyles, etc.).

Photography

There are two aspects to photography in our kind of study: first, the need to preserve an unambiguous record of the morphology of the bone before it is sectioned (in combination with the formal documentation mentioned in the preceding paragraph); and second, to compile a photographic record of the end result of the sectioning.

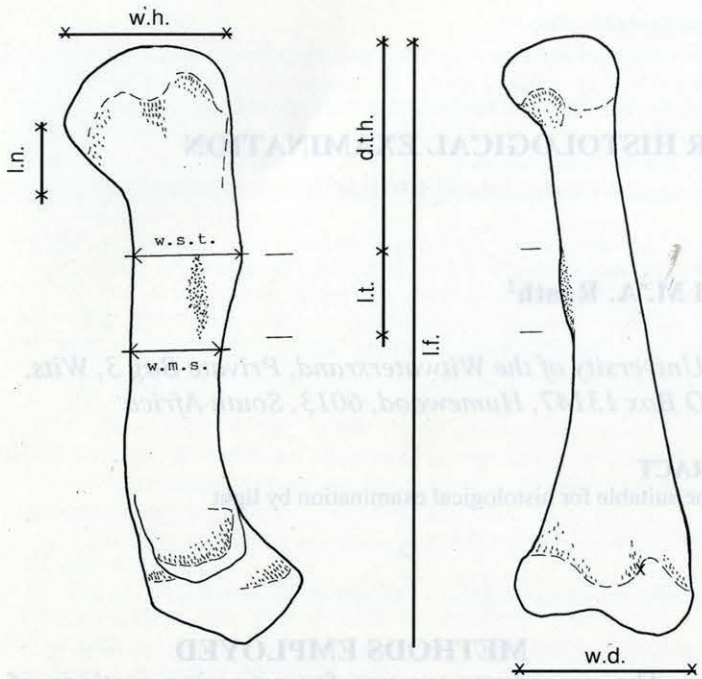


Figure 1: Suggested measurements which should be recorded prior to sectioning.

For the sake of repeatability in both instances, a detailed record of the photography should be kept and should preferably include all relevant technical details such as; film type and speed, camera settings (shutter speed, aperture, lens type, etc.), an indication of the section or slide number, the magnification used, whether or not crossed-nicols or other light-modifying accessories were used, and the light intensity.

Stored with all other relevant notes on the material used, these records become potentially useful archival resources for later reference.

Embedding of specimens for sectioning

Since fossil bones are, in many cases, relatively brittle, they often need to be embedded in resin or some other suitable mounting medium to reinforce the structure of the tissue and prevent them from shattering or disintegrating during the processes of sectioning and grinding.

We use a cold-curing epoxy resin, Epofix, but any resin or other rigid, clear mounting medium which does not interfere with the structure or optical properties of the tissue could be used.

The manufacturer's specifications should be followed meticulously when preparing the resin for use, especially with regard to the mixing proportions for the resin and its hardener; incorrect proportions can lead to disastrous results, including the generation of large amounts of heat during the polymerisation phase which can result in catastrophic damage to the specimen.

Any suitable disposable containers can be used in which to embed the bones in resin; in our laboratory we often use cheap plastic containers of various shapes and sizes. Care should be taken as many plastics are melted by the resins used in the embedding process, but polymerisation of the resin is generally rapid enough for this not to pose a major problem. Experience will soon indicate what kinds of plastic or other containers are unsuitable.

Where bones of a particular size range are often dealt with, it might be advisable to use custom-designed moulds in which to embed the bones. Moulds made of latex or silicone rubber have been found to work well.

To avoid wastage of resin, and also to facilitate easier handling of the specimen, the container chosen should have just sufficient room for the specimen, with not much to spare - about 0.5 - 1cm all around is enough. Prior to embedding the specimen itself, we advise that a layer of resin about 0.5 - 1cm thick be poured into the base of the container and allowed to harden in order to ensure that the bottom of the specimen is well embedded.

Since irritant fumes are generally given off by the resins used, the embedding process should take place in a chemical fume cupboard with the exhaust fan running.

Once the bone is embedded it should be left for the resin to cure until it is hard enough to be removed from the mould (up to about 24 hours, depending on the resin used). Best results are obtained if the embedding takes place under vacuum, as this helps to liberate trapped air bubbles and aids penetration by the resin.

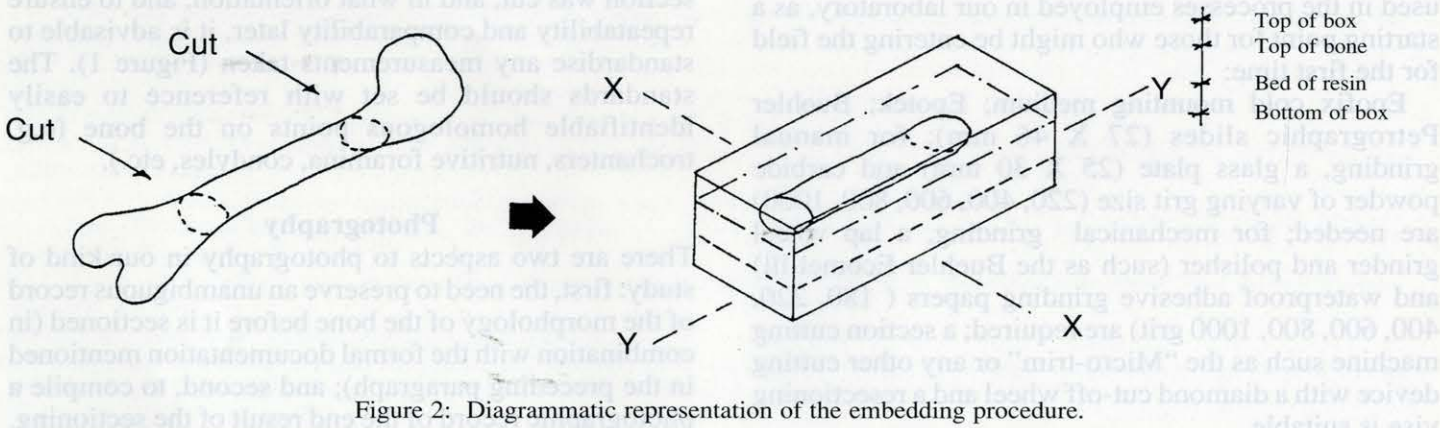


Figure 2: Diagrammatic representation of the embedding procedure.

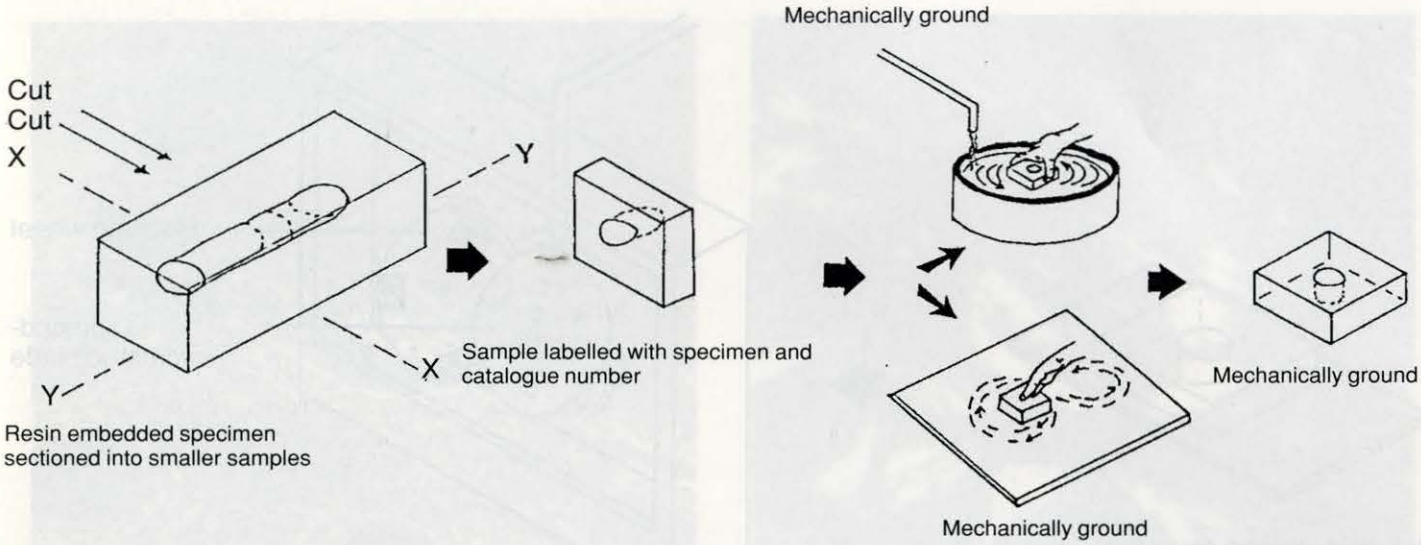


Figure 3: Sectioning of embedded bone and subsequent grinding of specimen.

Sectioning embedded bone

A surprising variation in bone tissue structure occurs in different bones of the same species, and even in different regions of the same bone. It is therefore important to know precisely where along the length of a bone a section has been cut and in what orientation (whether transverse, longitudinal, tangential etc). For this reason, in documenting the sections taken, it is helpful to relate the site of the cut to the standardised measurements of the bone advocated earlier. This facilitates repeatability of observations.

A short stub of bone is cut from the parent specimen using a suitable rock-cutting saw. In our experience a rock-cutter with a water-cooled diamond-studded blade works well. The dimensions (thickness and diameter) of the stub are not critical, but are essentially dictated by what the section cutting machine can accommodate and the size of the glass slides available on which the section is to be mounted; the section should not protrude beyond the edges of the slide.

Each resulting segment of the parent specimen is carefully labelled with its unique specimen number and basic catalogue reference, so that there can be no mistake about its identity and so that it can be easily reunited with its registration details. The way in which the segments are labelled will depend on a variety of factors, not least of which will be the size of individual segments. If the segment is large enough, the catalogue number can be written directly onto it using indelible ink (indian ink) on a small patch of white or other pale paint.

We have found exterior grade PVA paint (e.g. Plascon "Wall-'n-All") an acceptable and durable base on which to write the catalogue number. A satisfactory and relatively permanent label can be produced by writing on good quality paper or opaque polyester foil in waterproof black ink (indian ink), and using Glyptal G1276 cement thinned in lacquer thinners both as an adhesive to stick the label to the specimen and as a protective coating and sealant. If "clear" epoxy resin is used, the specimen and catalogue numbers can be written in pencil on white paper and set in the resin during the embedding.

Grinding one surface to affix to slide

The cut surface is ground down on successively finer abrasive grinding discs, until the surface is completely flat, smooth and free of scratch marks. Grinding can be carried out either manually or mechanically.

The procedure for grinding by hand is both time-consuming and laborious. It involves smoothing the rough cut surface by rubbing it on a glass sheet coated with an abrasive paste. Carborundum powder, in decreasing particle size - e.g. 220, 400, 800, and 1000 grit) - has produced acceptable results in our work. The grinding is carried out by small circular motions, and hand-pressure must be applied to the specimen evenly or wedging can result.

The mechanical procedure is much quicker and more efficient, since it reduces the amount of wedging that may occur, and fairly even surfaces are obtained. The apparatus consists of a rotating lap-wheel onto which waterproof abrasive grinding discs are fixed. Many different models of grinders are available. The grinding discs must be used in a sequence of decreasing coarseness (i.e. increasing grit figures); the sequence of discs we use is 180 grit, 220, 400, 600, 800 and finally 1000 grit. Aluminium oxide powder (0.3 - 0.05 μm) mixed with water is sprinkled onto a cloth-covered lap-wheel for final polishing. Aluminium oxide is also available in paste form, and we have used it sucessfully to obtain a high polish.

After the final polishing, the specimen is washed with water and dried. Excess moisture is removed by placing the specimen onto filter paper or paper towels, thus hastening the drying process.

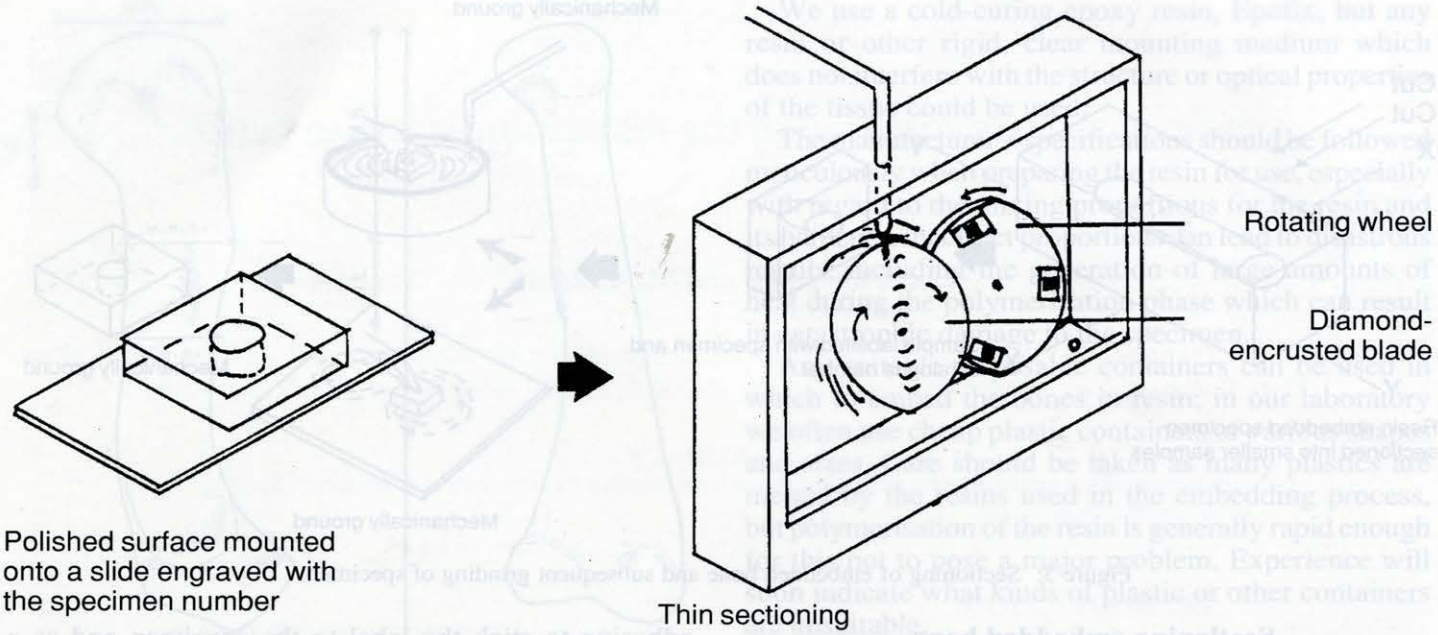


Figure 4: Diagrammatic illustration of the thin sectioning procedure.

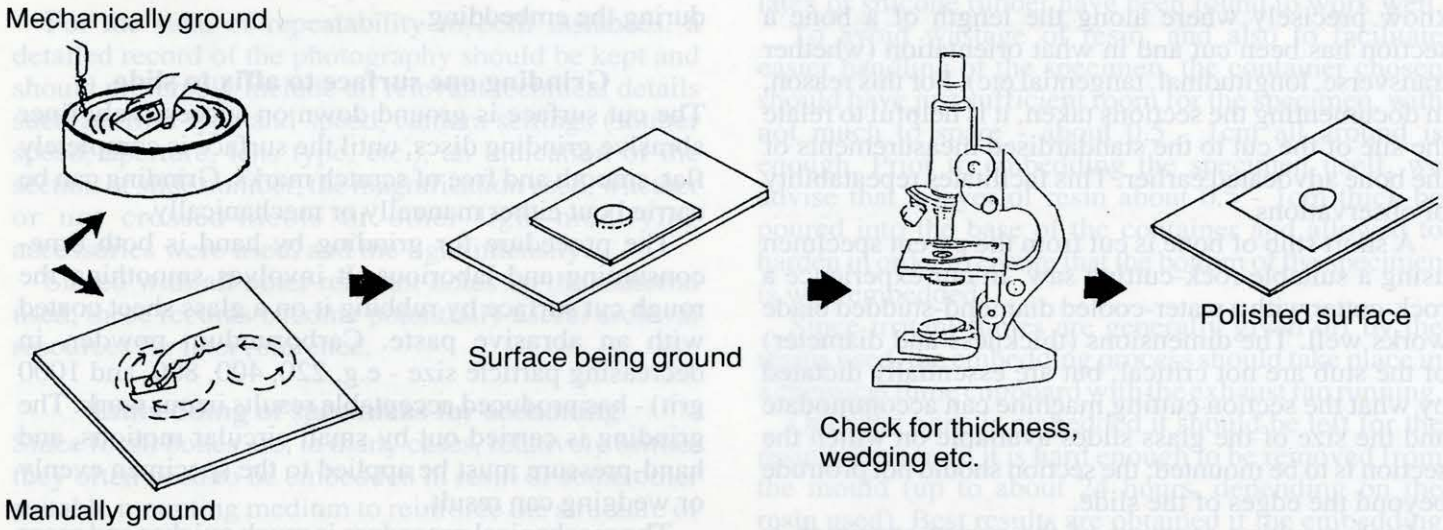


Figure 5: Grinding and polishing of specimen.

Mounting and labelling the stub

The polished surface of the stub of bone is then mounted onto a petrographic glass slide. In our experience frosted slides provide better optical resolution, as well as a better adhesive surface for the mounting medium; clear petrographic slides can be frosted by rubbing them gently on a glass plate with an abrasive paste of 400 grit carborundum powder.

In order to ensure that the slide to which the specimen is affixed remains identifiable during the process of section cutting and in storage afterwards, it must be labelled in some durable way. Indian ink and adhesive labels can be used, but we recommend that the specimen numbers be permanently engraved onto the glass slides using a diamond tipped stylus. We suggest that this be done before the polished specimen is mounted onto the slide since the slides might occasionally break or crack whilst being engraved, which renders them effectively useless. Clearly, if a slide is labelled before a specimen is stuck to it, care must be taken to ensure that the correct stub is stuck to the correct slide. A resin adhesive such as Epotek is used to fix the ground and polished end of the stub to the frosted slide.

The next step is to trim away excess material from the free end of the stub by cutting off slices approximately 1mm thick. This reduces the amount of coarse grinding that will be required. For our section cutting we use a "Micro-Trim" section-cutting machine, which automatically feeds the slides (held by vacuum on a rotating chuck) onto a water-cooled, rotating, diamond-encrusted blade.

Thin sections can also be made satisfactorily using a

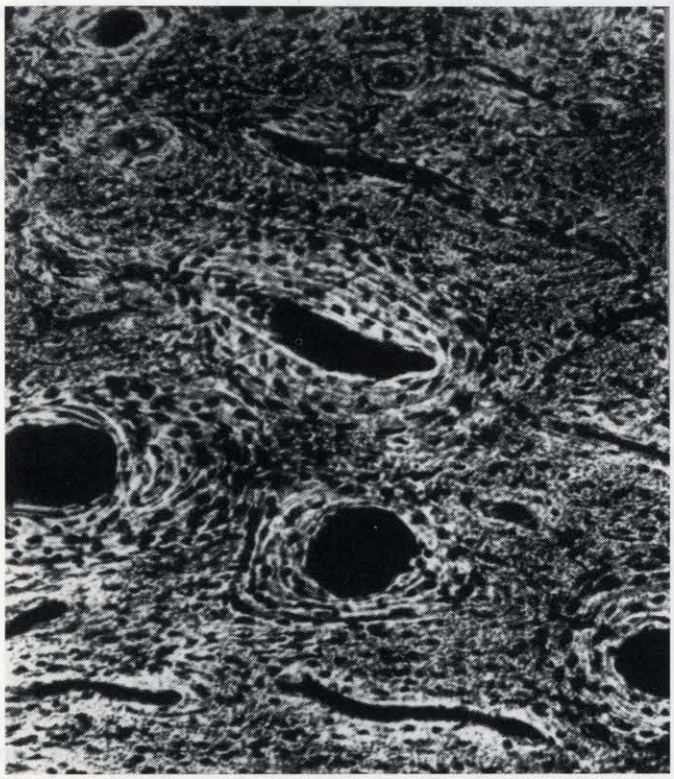


Figure 6: Transverse section of a humerus of the dicynodont *Kannemeyeria* showing primary and secondary osteons in a woven bone matrix. x490. BPI Palaeontlogy, specimen number H2.



Figure 7 Tissue shown in Figure 6, as seen with cross polarisers. X410 BPI Palaeontology, specimen number H2.

variety of other machines and devices, one of the simplest being a diamond cut-off wheel and a resectioning vise.

Finishing

The resulting thin sections will bear distinct cut marks across the free surface, which must be removed by carefully grinding as outlined above. Grinding continues until the cut marks have been removed, the surface is smooth, and the section is of acceptable thinness ready for final polishing. At intervals during grinding the slides should be examined under a low-power microscope to check thickness, amount of wedging or other distortion, and the amount of tissue detail visible (Figure 5). Depending on what is being studied the final thickness of the specimen can vary from about 120 µm to 20 µm (the latter thickness is adequate for histological purposes).

The finished slide is checked to ensure that it is correctly and adequately labelled. We recommend that it be stored together with any left-over fragments or portions of the parent specimen.

Gross microscopic examination of the prepared slide can initially be carried out under low power, e.g. through a dissecting stereomicroscope, to obtain overall information about gross structural features of the tissue More detailed examination is carried out under higher magnification (Figures 6 and 7).

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