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for the

PREPARATION OF GROUND SECTIONS FOR THE MICROSCOPY OF BONE TISSUE

G.J.R. Maat , R.P.M. Van den Bos and M.J Aarents

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INTRODUCTION

It is becoming increasingly pressing for osteoarchaeologists, paleopathologists and forensic anthropologists to have light microscopy as a diagnostic tool within easy reach. Because bone tissue seems so hard to prepare, it is understandable that one tries to avoid the use of microscopy as an aid for the determination of age at death, the diagnosis of diseases, the assessment of bone tissue decomposition, mineralization and metabolism. Refraining from microscopy is often based on the widely spread misunderstanding that the preparation of ground sections is expensive, demands a lot of time and sophisticated instruments, knowledge of plastics and stamina to face failed attempts to produce a useful section.

As early as in 1958, Frost recommended an elegant procedure for the "preparation of thin undecalcified bone sections by rapid manual method" (Frost, 1958). The technique was developed for the processing of fresh bone tissue "to provide a means for the microscopic observation of bone that appears close to the ideal of observation *in vivo*". To our entire satisfaction we have also applied Frost's original technique for many years on regular dry bone tissue of moderate to good preservation. It has proven to be very cheap and reliable. With time we made and tested some modifications that further improved the technique and which also made it very suitable for less well preserved inhumed and even cremated osteoarchaeological and forensic material. As in the original method the modifications need only a few extra but still very basic and cheap products.

MATERIALS

Bone material

To illustrate various steps in section preparation for this manual, bone material is used from a random inhumation of the Late Medieval Period (Maat and Mastwijk, 2000) and from a cremation of the second century AD (Van den Bos and Maat, in preparation). They are chosen to cover a wide variety in bone preservation and tissue texture. Preservation of the inhumation was moderate. The skeleton was brownish discolored and incomplete. In general, the periosteal surface did not come off. Its dry bone tissue seemed to be firm. Transverse sections were made of the anterior part of the femoral shaft (mainly compact bone), of a piece of the parietal bone (compact outer and inner table weakly interconnected by trabecular diploë) and of a rib (thin cortical shell of compact bone with a delicate lattice of trabeculae having little coherence). The cremated remains were severely fragmentized and heavily calcinated, sometimes charred internally. The fragments were porous and very fragile.

Equipment

Apart from the microscope, all materials needed for the preparation of sections were readily available from hardware store and histology lab. Necessary are:

--- **A regular light (bright-field) microscope**, preferably with a polymerization filter set. If the latter is not available, you can cut both filters from a sheet of plastic Polaroid filter or from plastic Polaroid sunglasses. One filter (the analyzer) is positioned into the tube of the ocular lens, the other (the polarizer) is put onto the light source of the microscope.

--- **A powerful magnifying glass** (10-15x) or a dissection/surgery microscope. After some experience you can do without!

--- **A small hacksaw** or electric band saw.

--- **A rectangular thick slab of glass**. At all sides it should be 1 mm shorter than the sheet of abrasive (sand) paper to be used. See below. In our case the size of the slab was 13.8 x 22.8 cm.

--- **Sheets of tough good quality waterproof carborundum paper**: grit Nr. 220. We used SEGRO®, SW-FL-Latex, P 220, size 23.0 x 28.0 cm. A sheet was cut into halves. Each half should always be a little oversized with respect to the glass slab (see above). For every section only half of a sheet is needed.

--- **Glass microscope slides** for routine histology. Most frequently we used the usual 76 x 26 mm size. The much larger 76 x 40 mm size produced even better results.

--- **Glass microscope coverslips** for routine histology. Most frequently we used the 24.0 x 40.0 mm size.

--- **A small soft artist's paintbrush**.

--- **Thin (non viscous) cyanoacrylate glue** ("super glue", the fluid type!).

--- **Mounting medium**. We used Entellan® from MERCK. Others, like Depex® will also do.

--- **Vaseline®**.

--- **Alcohol** of a high percentage, e.g. 90%.

--- **Xylene**.

--- **Kitchen detergent** (fluid soap), e.g. Teepol®.

--- **Tap and distilled water**.

METHOD

The following step-by-step description of the technique is drawn up for the preparation of regular sections of bone of reasonable consistency, in the first place. They don't need intrinsic strengthening. Since the description is detailed it seems that section preparation will take much time, but in practice it takes less than 20 minutes.

Additional instructions for fragile pieces of bone that do need intrinsic strengthening to prevent damage during the preparation process (cremations and specimens with a lot of cancellous bone) are printed in italics.

Step 1: Cut a sheet of waterproof abrasive paper neatly into halves with an old pair of scissors. Grease the glass slab thinly and evenly to its edges with Vaseline®, and stick one of the halves onto the slab with its abrasive side up Figures 1, 2 and 3.

The waterproof sheet should be a little oversized with respect to the glass slab to prevent water from reaching its back. If the back of the sheet becomes wet it will ripple and become useless. Make sure that no dirt or air gets trapped between the glass slab and the abrasive paper. A minute grain under the abrasive sheet will ruin a thin section during grinding. If the greased glass slab stays clean, a fresh sheet for the next section can be positioned directly on the slab after some additional greasing.

Step 2: Cut off, by means of two parallel cuts, a ca. 2-3 mm thick slice of bone with the hacksaw or the electric band saw. Figure 4.

Let the hacksaw do the work, instead of applying force. If you use an electric band saw, avoid heat development. Do not scratch the periosteal surface if you are preparing a section for an osteon count for age at death determination!

In case of a fragile specimen: take in mind a thicker slice of bone than is usually done for regular sections i.e., 4-5 mm. To prevent tissue damage during the preparation, apply a few drops of cyanoacrylate glue before cutting onto the periosteal surface that you intend to cut and let it harden (Figure 5). After you made the first cut, do the same with the surface made by this first cut (Figure 6). If many drops are applied with the intention of immersion, let it harden for 24 hours. Cut off the slice you had in mind and drop sufficient cyanoacrylate glue onto the surface made by the second cut. Let the slice harden on a piece of paper. Later on, the paper will easily wear down and pulverize during the following grinding process.

Step 3: Moisten the central area of the abrasive paper with tap water, add a few drops of Teepol® if the slice is greasy as sometimes is the case in fresh and forensic specimens, and grind both sides of the thick slice by hand with a rotating motion until both sides are smooth and flat (Figure 7). Apply moderate pressure during grinding. Do not let the slice topple.

One of the reasons for doing this in the central area of the abrasive sheet is to reduce its sharpness. At the same time the slice gets flat and level sides for a firm hold by Frost's gripping device during the following processing.

In case of a fragile specimen which has been strengthened by cyanoacrylate immersion: after the described first grinding of both sides of this extra-thick slice, finish (polish) one of the two sides by applying light to medium pressure on its center during the rotating motion. Do this in the blunt central area of the abrasive sheet to produce a very smooth and completely flat and level surface! One may check the result with the help of the magnifying glass or the dissection/surgery microscope. Subsequently, clean the finished side with distilled water with detergent and let it dry. Stick the finished side with cyanoacrylate glue to the center of a new and alcohol cleaned glass microscope slide. The best way to do this is by putting the glass slide onto a supporting piece of thick glass that will stay flat under pressure. Apply a few drops of glue on the center of the glass slide (Figure 8). Place the thick slice of bone with its polished side down immediately in the wet glue on the slide (Figure 9). Put a strip of cardboard and a weight with its flat smooth side down directly on top of it and let the glue harden for two hours only (Figure 10). After two hours the hardening glue is still sufficient sticky to hold the section onto the glass slide during wet grinding. After a stay overnight it may have lost too much flexibility and the section may come off during further grinding. Do not let the specimen remain in this phase for days. Shrinkage of the hardening cyanoacrylate may shatter the glass slide. The strip of carton prevents the weight from adhering to the specimen. Later on, the cardboard will easily wear down and pulverize during the following grinding process.

Step 4: "Frost's gripping device", the so-called section holder, is put together by folding a slip of fresh abrasive paper, with its abrasive side outward, transversally across the central part of one side of a glass microscope slide. The two free ends of the slip are used to hold the device. This is done by putting the index finger in between the free ends on the center of the glass slide and by positioning the thumb and middle finger of the same hand on the outward side of the same ends (Figure 11).

The width of the slip of abrasive paper should be amply larger than the size of the slice of bone. Since its abrasive surface is fresh and sharp, it will easily hold the slice/section after applying pressure during the rotating motion in the central area of the more blunt abrasive sheet. In this area friction between the slice of bone and the abrasive paper has become considerably reduced due to blunting during step nr.3.

Step 5: Wear down both sides of the slice alternately by applying light to medium pressure with Frost's device, the holder, onto the section during the rotating motion (Figure 12). Start in the central area of the sheet where the sheet is less sharp and try to work your way gradually to the sharper periphery. Avoid coming near the edges of the sheet where it might be contaminated with traces of Vaseline® or might be turned up in a minute degree (invisible to the naked eye).

Always use a soft brush, instead of tweezers, to turn the section over from one side to the other during alternation (Figure 13). Add some tap water if necessary for grinding. Rinse off the grinding surface above the sink if it becomes too "milky" or if you feel a grain. After a short period of about 10 minutes, the regular dry bone slice will have been be reduced to a thin section. In case of fresh tough bone tissue this may take 5 minutes longer.

In case of a specimen which has been glued to the center of a glass microscope slide (see step nr. 3): use the glass microscope slide itself as a holder to wear down the thick slice of bone. If it is much too thick or lumpy, a lot of time can be saved by first removing excess bone parallel to the glass with the hacksaw, the electric band saw or with rough abrasive paper. To hold the glass slide during the rotating motion, moisten your index- and middle finger a little and place them onto the center of the glass slide (Figure 14). To avoid minute distortion between section and glass slide apply most pressure straight above/opposite the section. Start the rotating motion in the central area of the abrasive sheet. Carefully increase the applied pressure. Get the feeling and go gradually to more peripheral areas.

Step 6: Grind down the section to its final thickness in the more or less blunt central area of the abrasive sheet. This is done when the thin section becomes opaque. Use of an extra abrasive sheet with a finer grid (e.g. P 1200) is not necessary. In general, the section is finished (and polished) when it has become so transparent that it isn't spotted at first sight (Figure 15).

In this phase of the process it is very useful for "beginners" to keep in touch with the reduction process by regularly checking the achieved thinness on the section holder. This is done by inspection with oblique light of all edges of the section from aside through the magnifying glass or the dissection/surgery microscope (Figure 16). Keep the section on the holder moist at all times during inspection. The thinner the thin section gets, the more effectively potential toppling of the gripping device is reduced by the larger glass slide during the rotation motion, and the more level the section becomes automatically. Sometimes, because of local differences in hardness/resistance within the section, a slight local thickness correction may be needed. The too thick part will show up as more "opaque". Corrections can be easily made during the rotation motion by (re-)placing the index finger on the glass slide to a spot above/opposite the site of correction.

In case of a specimen which has been glued to the center of a glass microscope slide (see step nr. 3 and 5): due to friction and the continuous presence of water during the final grinding, the glued section may come off from the glass slide. If so, don't worry, proceed, and just process it as a regular section. Do not remove the flange of hardened glue around the section. Fit together a Frost's device (step nr. 4) and continue only to grind the unfinished side of the section until it is finished too. Do not turn the section over!! The other side, which was glued against the holder, was already finished and will stay intact in the embedding substance of cyanoacrylate glue as long as you don't grind it off.

Step 7: Always keep the final section wet to prevent it from curling. Clean it by submersing in distilled water with a few drops of detergent (Figure 17). Use the small soft brush with care to brush and to turn over the section underwater (Figure 18). Cleaning underwater keeps the section in a state of suspension and avoids abrupt movements. Refresh the distilled water and repeat the cleaning. Careful rinsing supported by a cleaned glass slide may give the final touch.

Step 8: Lift the cleaned section out of the water with the help of the soft brush, and put it on a piece of filter paper in a Petri dish to dry. The drying period can be reduced substantially by rinsing the section in alcohol 70% or higher. Use rumpled filter paper to facilitate the lifting of the dried section during the next step (Figure 19).

Step 9: If requested, the regular unembedded dry section can now be used for any histological staining by putting it into a porcelain-filtering cup and using this as a carrier. Since there is no embedding material, staining time should be kept relatively short if compared to routine paraffin embedded sections.

Step 10: Clean a glass microscope slide with alcohol and put it on a thick glass slab with some dark paper underneath (background). Apply a few drops of mounting medium on its center (Figure 20), lift the dried section out of the Petri dish by lifting it up on the tips of a pair of pointy tweezers and put it on top of the drops as quick as possible (Figure 21). Immediately add a some more mounting medium onto the section (Figure 22). Check weather the section is still in the center of the slide. If necessary correct its position by pushing it with a needle. Without waiting, dip a glass coverslip completely in xylene (Figure 23), remove excess xylene by tapping one of its corners against filter paper, and lower it gradually over the immersed section (Figure 24, 25 and 26 and the paragraph on trouble shooting). The mounting medium will spread itself underneath the slip. When ready and if requested, transport the slide carefully in a horizontal position for immediate microscopic inspection of the section. Let the specimen rest for a day in a horizontal position before storing it in a special box for microscopic slides.

In case of a fragile specimen which is still glued on the center of the glass microscope slide (see step nr. 3 and 5): dip the glass slide together with the section into the xylene. Remove excess xylene by tapping one of its corners against filter paper. Put the glass slide with the section up on a thick glass slab with some dark paper underneath (dark background). Immediately add a few drops of mounting medium onto the section, and cover it with a glass slip as described above in step nr. 10. Remaining traces of xylene will be of help to immerse the section with mounting medium i.e., to fill in all "vacant" places.

TROUBLE SHOOTING:

Air bubbles under the glass coverslip.

Trapped air above the bone section happens if the glass coverslip was not lowered properly during final mounting (see step nr. 10). Trapped air can be easily detected against the dark background of the thick glass slab. Its occurrence is a typical annoyance for non-routine processors and has nothing to do with the preparation of a perfect section. Because many workers are not familiar with the proper way to lower a glass coverslip, the following instructions may be useful:

1. Dip a coverslip completely in the xylene with the help of flat tipped tweezers (Figure 23),
2. Take two nearby corners of the coverslip between thumb and index finger,
3. Remove excess xylene, not all, by tapping one of the two free corners against filter paper,
4. Lower that corner and keep it onto the glass microscope slide and let it make contact with the drops of mounting medium around the section,
5. Drag that corner with some mounting medium over the glass slide to its final position (Figure 24),

6. In addition lower the other free corner slowly to its final position on the glass slide (Figure 25),
7. Lower the other end of the glass slip slowly over the immersed section by supporting that free side on the tips of the pointy tweezers. At the same time prevent the glass slip from slipping away by positioning a fingertip of the other hand onto the glass slide against the other (wet) end of the slip (Figure 26).

There are two ways to remove air bubbles:

--- The most radical way is to put the complete specimen upright in a container with xylene until the coverslip and section has come off by gravity. In case of a fresh specimen this only takes minutes. If the embedding medium is completely hardened after many days this takes an overnight. Remount the section while it is still wet from the xylene on a fresh glass slide, or:

--- Put the complete specimen on a thick glass slab, put a piece of paper which is just smaller than the coverslip on top of the slip, and put the smoothened side of an adequate metal weight onto that paper. Scratch a little with a sharp object somewhere along the edge of the coverslip so that excess mounting medium can ooze or bleed out (Figure 27). This will make the coverslip lower onto the section and thus dislodge all air bubbles above the section. Leave it for a day. If by that time the weight has got stuck to the specimen, just let some xylene suck into the piece of paper with the help of a pipette and after a short while the weight will come off (Figure 28). And in case the glass slide itself has got stuck to the thick glass slab, let some xylene flow in between with the help of the same pipette (Figure 29). After a short while the slide will come off too.

Too thick section.
Remove the section for additional grinding by putting the complete specimen upright in a container with xylene until the coverslip and section comes off by gravity. In case of a fresh specimen this takes only minutes, if the embedding medium is completely hardened this may take an overnight. Clean/rinse the section with xylene and let it dry. Restart with step nr.6.

Section of uneven thickness.
Same procedure as in case of a "too thick section" (see above), but take special care of the recommendations on "local thickness corrections" in step nr. 6.

Broken section.
It takes more time and effort to horse around with a broken section than to produce a new one.

"Dirty", "smudgy" specimen.
Specimens processed according to the description above, will be perfectly clean. If so, then the embedding medium (hardened cyanoacrylate glue and/or mounting medium) surrounding the section should be clean too (check). Sometimes during microscopical inspection the section itself seems to be dirty. In those cases this is always caused by ante-processing factors. The three far most common causes for this are:

--- Body fat remains. Especially in specimens from forensic cases remaining body fat may give a smudgy effect in the finished section. This can be easily solved by removing the section like in case of a "too thick section" (see above). Leave the section in xylene for 1-24 hours until it is completely clear. Restart with step nr.10.

--- Postmortem fungal invasion. This will show as "dirty" focal microscopic tunneling of a uniform caliber spreading out from the periosteal and/or endosteal surface or from the interior of the vascular canals of Havers and Volkmann (see for instance Hackett, 1981).

--- Recrystallized apatite from the bone matrix (Herrmann and Newesely, 1982) and crystalized soluble minerals from the soil and ground water (Behrensmeyer, 1978). Since these minerals were not layed down in an organic way like from growth or remodelling, they were deposited as an amorphous substance dispersing the light of the microscope. This produces an annoying hazy effect. It makes the section look "too thick". Further grinding will not be of any help.

DISCUSSION

As one will experience with the manual method described above, the preparation of regular ground sections for the microscopy of natural, osteoarchaeological and forensic bone tissue is easy, cheap, rapid and produces beautiful sections (Figure 30, 31, 32 and 33). Since no embedding resins are used, they cannot interfere with routine and special histological staining, for example those based on interactions with enzymes and immune derivatives. Especially for forensic diagnoses of age at death its reliability and speed is most rewarding.

ACKNOWLEDGEMENTS

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FIGURES

Figure 1: Cutting a sheet of waterproof abrasive paper into halves.

Figure 2: Greasing the glass slab with Vaseline®.

Figure 3: Sticking one of the halves of abrasive paper onto the glass slab.

Figure 4: Cutting, by means of two parallel cuts, a ca. 2-3 mm thick slice of bone.

Figure 5: In case of a fragile specimen, apply a few drops of cyanoacrylate glue ("super glue") onto the periosteal surface before cutting.

Figure 6: In case of a fragile specimen, apply also a few drops of cyanoacrylate glue onto the surface made by the first cut.

Figure 7: Grinding both sides of the thick slice by hand with a rotating motion.

Figure 8: In case of a fragile specimen, apply a few drops of glue on the center of the glass slide.

Figure 9: Placing the thick slice of fragile specimen with its polished side down immediately in the wet glue on the slide.

Figure 10: A fragile specimen which has been strengthened by cyanoacrylate immersion has been stuck with its finished side onto a glass microscope slide. Note the supporting piece of thick glass, the thick slice of cremated bone, the strip of cardboard and the weight.

Figure 11: How to hold "Frost's gripping device", the so-called section holder.

Figure 12: Wearing down both sides of the slice alternately by applying pressure with the section holder onto the section during the rotating motion.

Figure 13: Using a soft brush to turn the section over from one side to the other.

Figure 14: How to use the glass microscope slide itself as a holder in case of a fragile specimen which has been glued to the center of a glass microscope slide.

Figure 15: In general, the section is finished (and polished) when it has become so transparent that it hard to spotted it at first sight.

Figure 16: Checking the achieved thinness by inspection with oblique light of all edges of the section from aside through the dissection microscope. The section stays on the section holder!

Figure 17: Putting the finished section into distilled water with a few drops of detergent.

Figure 18: Using the small soft brush to brush and to turn over the section underwater.

Figure 19: The cleaned section has been put on a piece of filter paper in a Petri dish to dry. Note the rumpled filter paper.

Figure 20: Applying a few drops of mounting medium on the center of a cleaned glass microscope slide. Note the thick glass slab with some dark paper underneath (background).

Figure 21: Putting the dried section, resting on the tips of a pair of pointy tweezers, on top of the drops of mounting medium as quick as possible.

Figure 22: Immediately adding some more mounting medium onto the section.

Figure 23: Dipping a glass coverslip completely in xylene with the help of tweezers.

Figure 24: Final position of the first lowered free corner of the coverslip after it dragged some mounding medium from around the section.

Figure 25: Now also the other free corner has been lowered to its final position.

Figure 26: The other end of the glass slip is slowly lowered over the immersed section by supporting that free side on the tips of the pointy tweezers. Note that at the same time a fingertip is positioned onto the glass slide against the other (wet) end of the glass slip to prevent it from slipping away.

Figure 27: Oozing/bleeding of excess mounting fluid along the edge of the coverslip in order to dislodge air bubbles above a section. Note the thick glass slab, the piece of paper that is just smaller than the coverslip and the metal weight.

Figure 28: In case a weight has got stuck to the specimen, let some xylene suck into the piece of paper with the help of a pipette.

Figure 29: In case the glass slide has got stuck to the thick glass slab, let some xylene flow in between with the help of a pipette.

Figure 30: Micrograph of a piece of human parietal bone (external table and diploë) from the Late Mediaeval Period. Section thickness: 11 micron. Bright-field. Microscope magnification 6.6 x.

Figure 31: Micrograph of a transverse section of a human rib from the Late Mediaeval Period. Section thickness 12 micron. Bright-field. Microscope magnification 6.6 x.

Figure 32: Detail of Figure 30. Polarized light. Microscope magnification 66 x.

Figure 33: Micrograph of a transverse section through the anterior part of a cremated and heavily calcinated human femoral shaft from the Roman Period. Polarized light. Section thickness 13 micron. Microscope magnification 66 x.

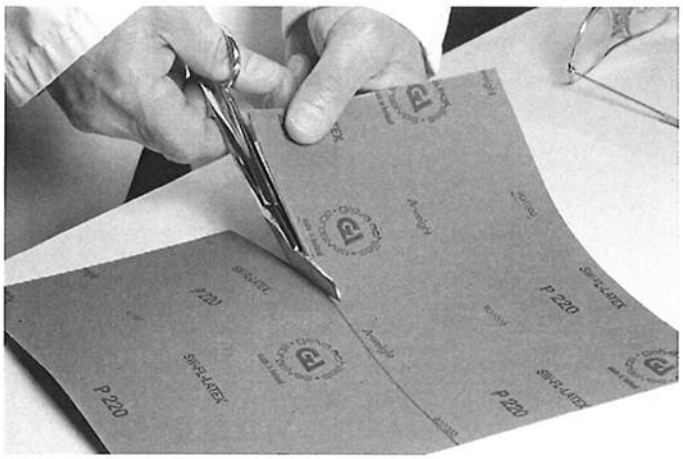

Figure 1: Cutting a sheet of waterproof abrasive paper into halves.

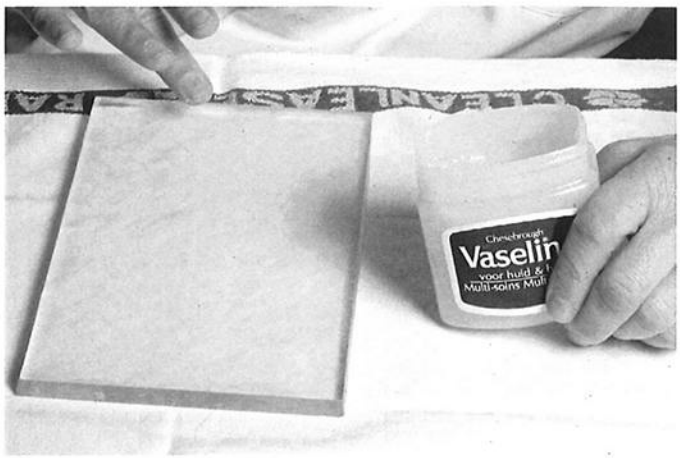

Figure 2: Greasing the glass slab with Vaseline®.

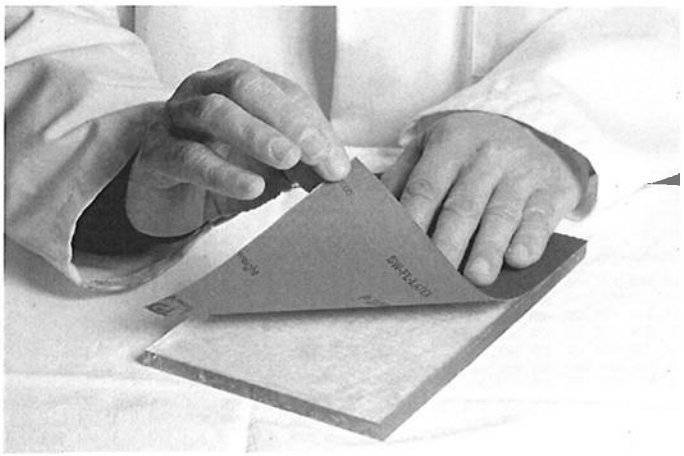

Figure 3: Sticking one of the halves of abrasive paper onto the glass slab.

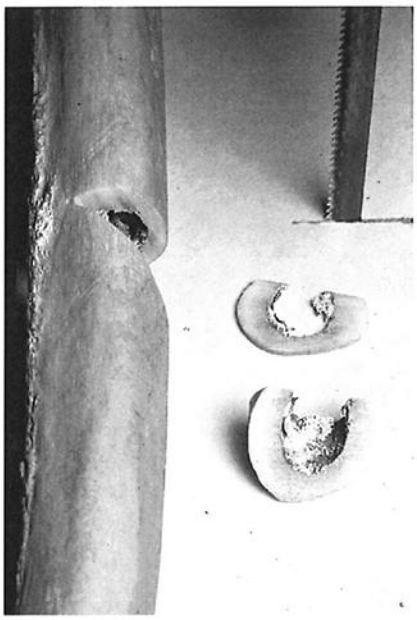

Figure 4: Cutting, by means of two parallel cuts, a ca. 2-3 mm thick slice of bone.

Figure 5: In case of a fragile specimen, apply a few drops of cyanoacrylate glue ("super glue") onto the periosteal surface before cutting.

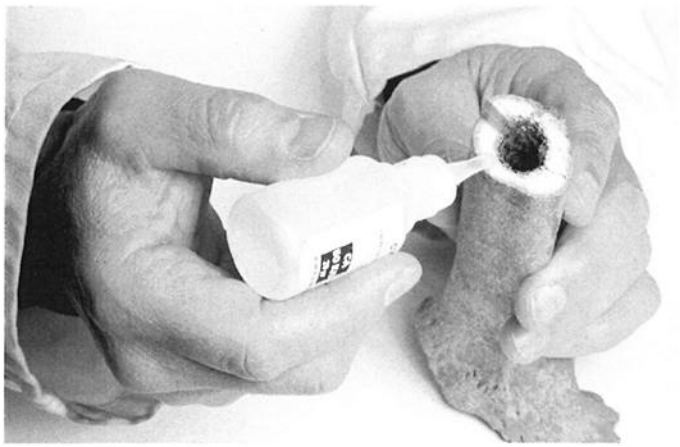

Figure 6: In case of a fragile specimen, apply also a few drops of cyanoacrylate glue onto the surface made by the first cut.

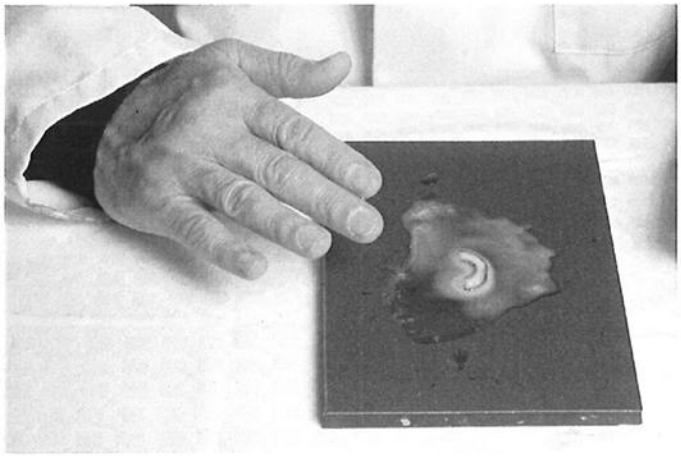

Figure 7:
Grinding both sides of the thick slice by hand with a rotating motion.

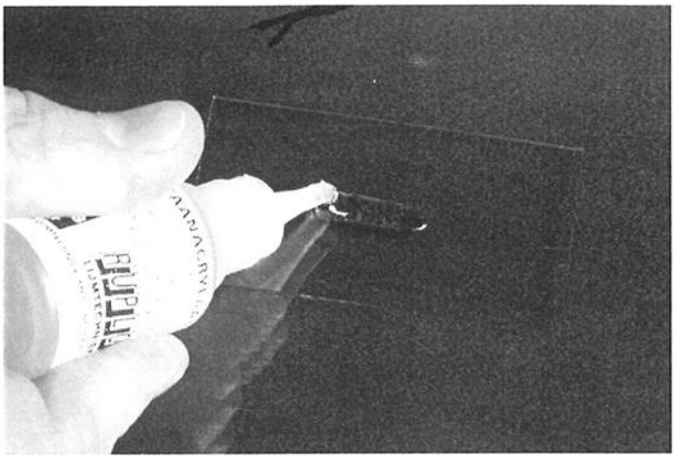

Figure 8: In case of a fragile specimen, apply a few drops of glue on the center of the glass slide.

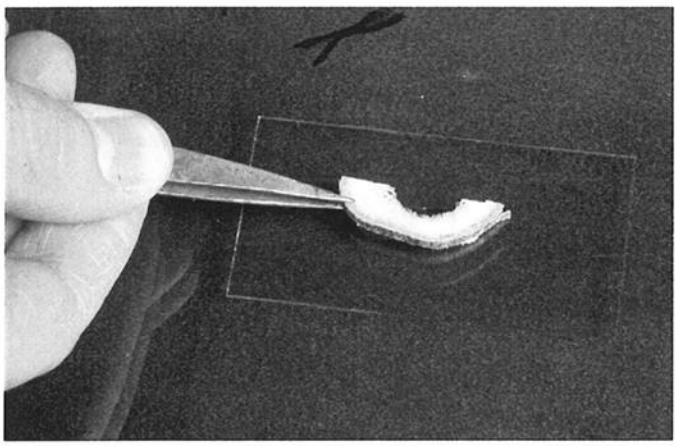

Figure 9: Placing the thick slice of fragile specimen with its polished side down immediately in the wet glue on the slide.

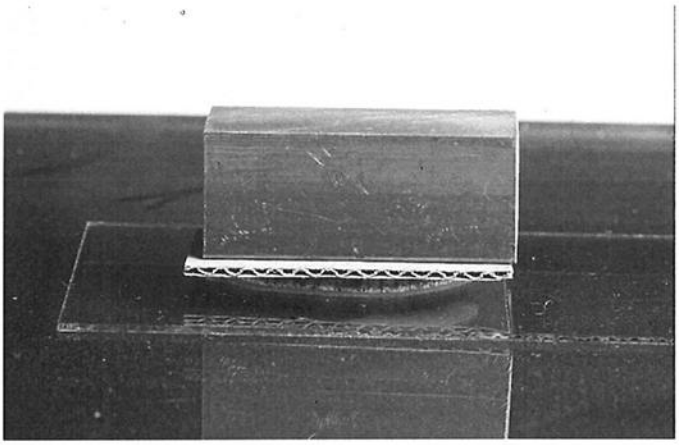

Figure 10: A fragile specimen which has been strengthened by cyanoacrylate immersion has been stuck with its finished side onto a glass microscope slide. Note the supporting piece of thick glass, the thick slice of cremated bone, the strip of cardboard and the weight.

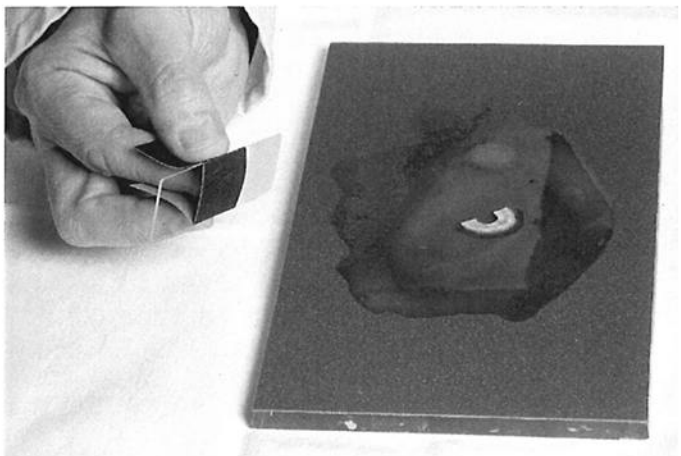

Figure 11: How to hold "Frost's gripping device", the so-called section holder.

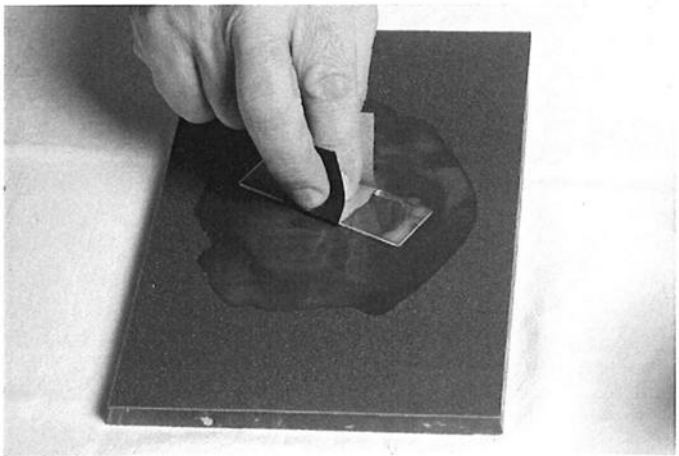

Figure 12: Wearing down both sides of the slice alternately by applying pressure with the section holder onto the section during the rotating motion.

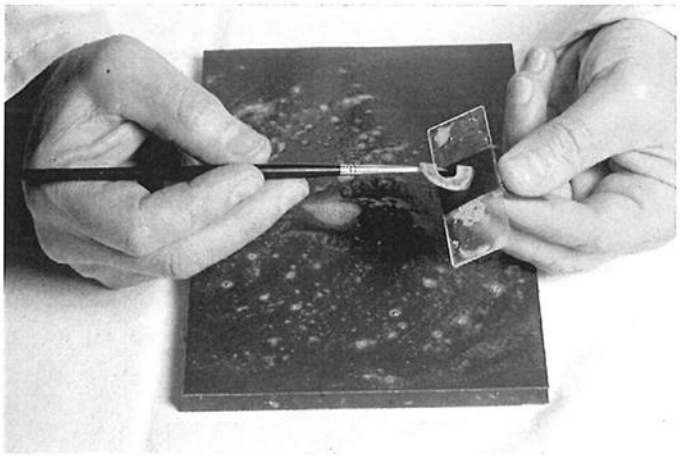

Figure 13: Using a soft brush to turn the section over from one side to the other.

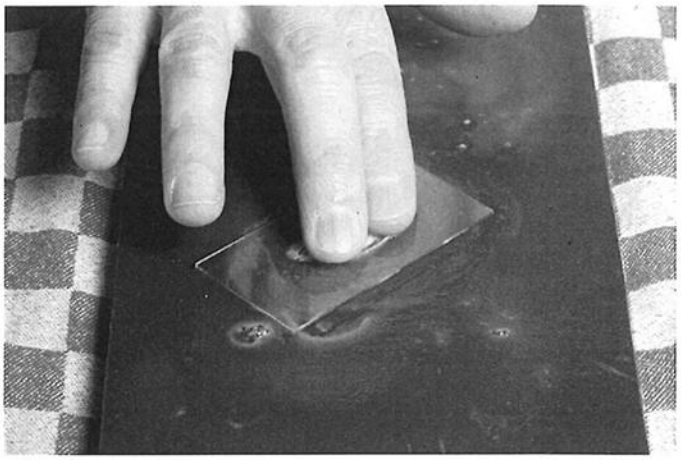

Figure 14: How to use the glass microscope slide itself as a holder in case of a fragile specimen which has been glued to the center of a glass microscope slide.

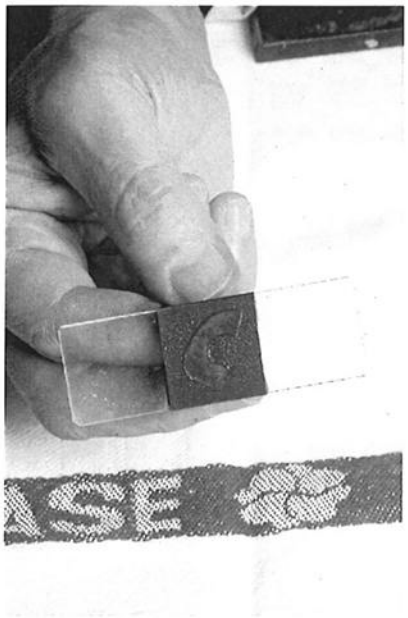

Figure 15: In general, the section is finished (and polished) when it has become so transparent that it hard to spotted it at first sight.

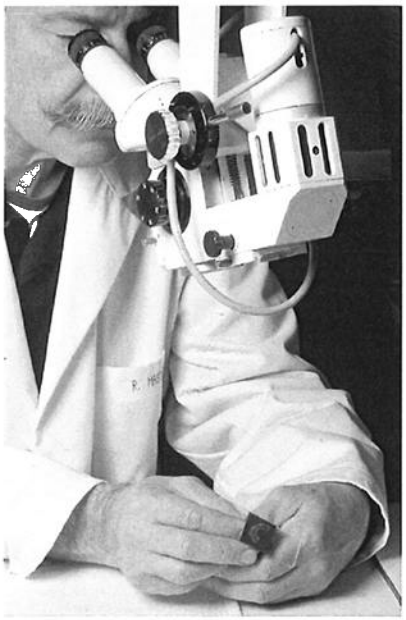

Figure 16: Checking the achieved thinness by inspection with oblique light of all edges of the section from aside through the a dissection microscope. The section stays on the section holder!

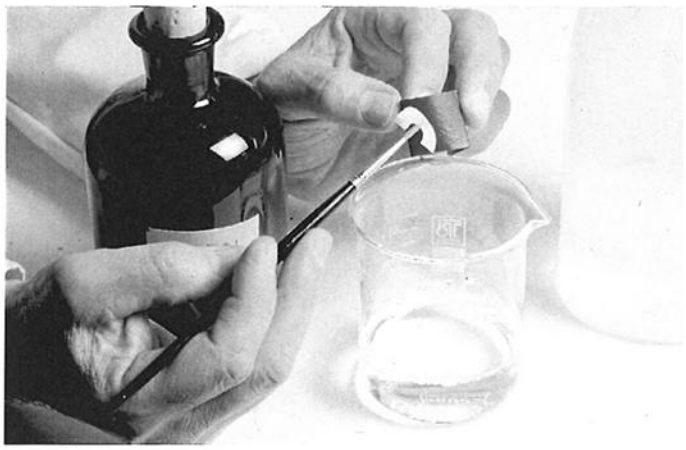

Figure 17: Putting the finished section into distilled water with a few drops of detergent.

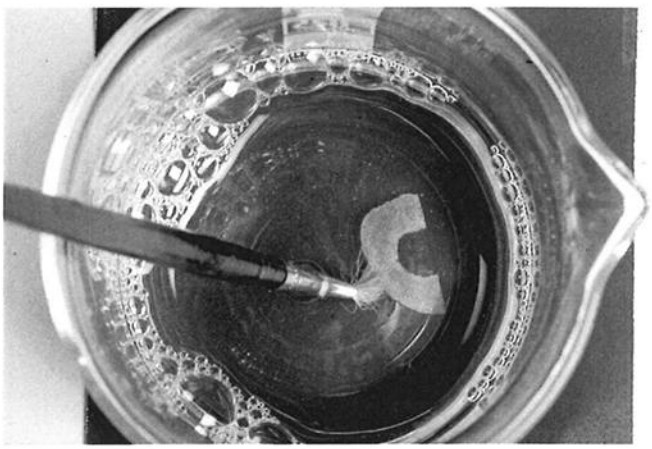

Figure 18: Using the small soft brush to brush and to turn over the section underwater.

Figure 19: The cleaned section has been put on a piece of filter paper in a Petri dish to dry. Note the rumpled filter paper.

Figure 20: Applying a few drops of mounting medium on the center of a cleaned glass microscope slide. Note the thick glass slab with some dark paper underneath (background).

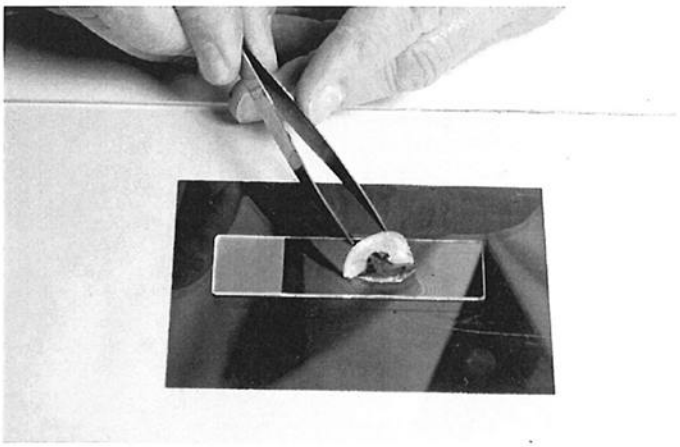

Figure 21: Putting the dried section, resting on the tips of a pair of pointy tweezers, on top of the drops of mounting medium as quick as possible.

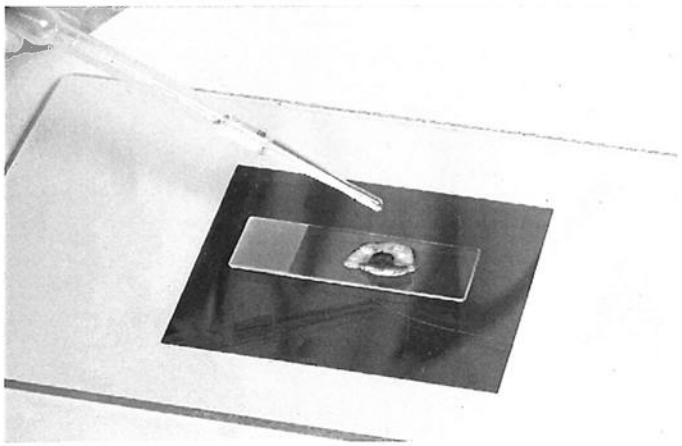

Figure 22: Immediately adding a some more mounting medium onto the section.

Figure 23: Dipping a glass coverslip completely in xylene with the help of tweezers.

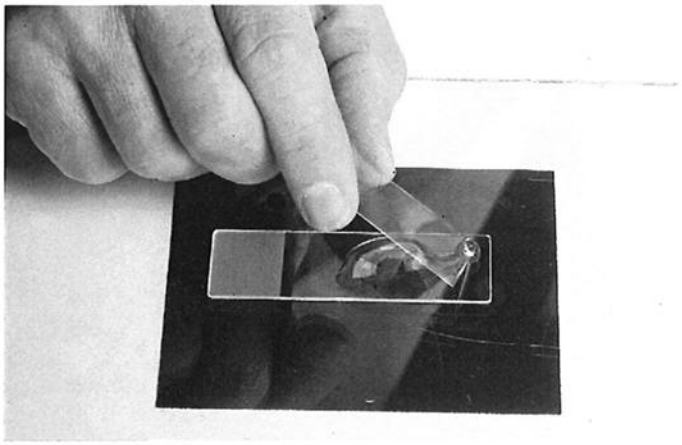

Figure 24: Final position of the first lowered free corner of the coverslip after it dragged some mounding medium from around the section.

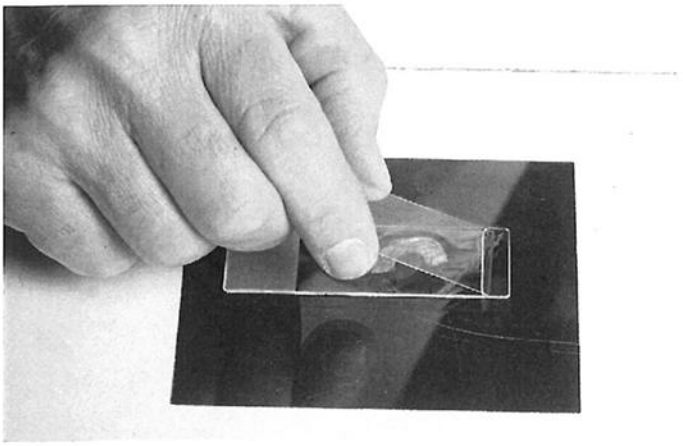

Figure 25: Now also the other free corner has been lowered to its final position.

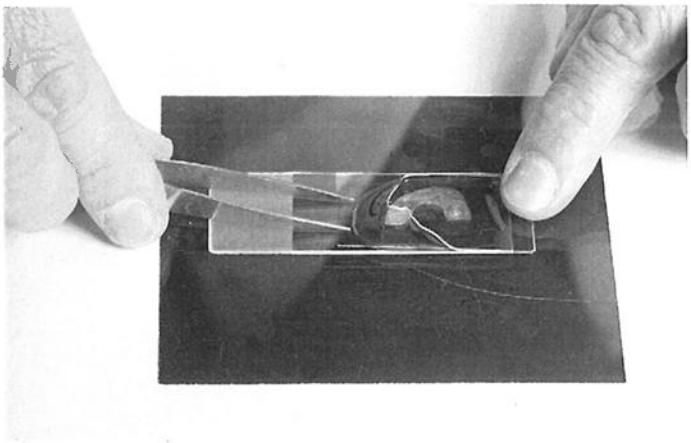

Figure 26: The other end of the glass slip is slowly lowered over the immersed section by supporting that free side on the tips of the pointy tweezers. Note that at the same time a fingertip is positioned onto the glass slide against the other (wet) end of the glass slip to prevent it from slipping away.

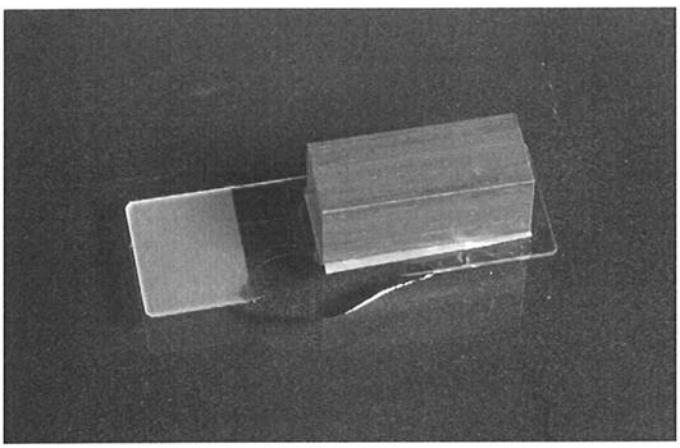

Figure 27: Oozing/bleeding of excess mounting fluid along the edge of the coverslip in order to dislodge air bubbles above a section. Note the thick glass slab, the piece of paper which is just smaller than the coverslip and the metal weight.

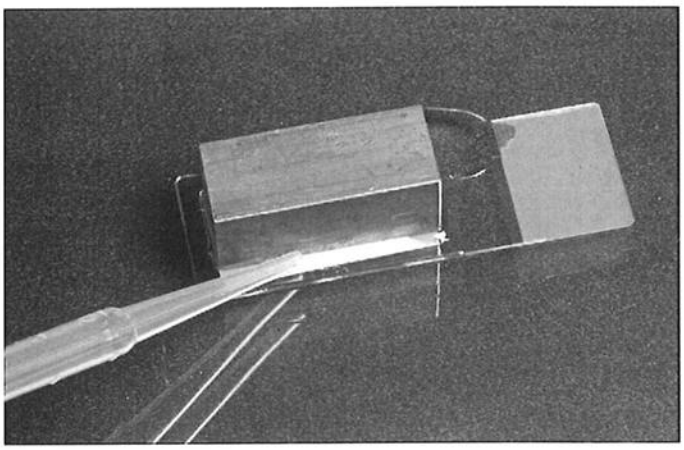

Figure 28: In case a weight has got stuck to the specimen, let some xylene suck into the piece of paper with the help of a pipette.

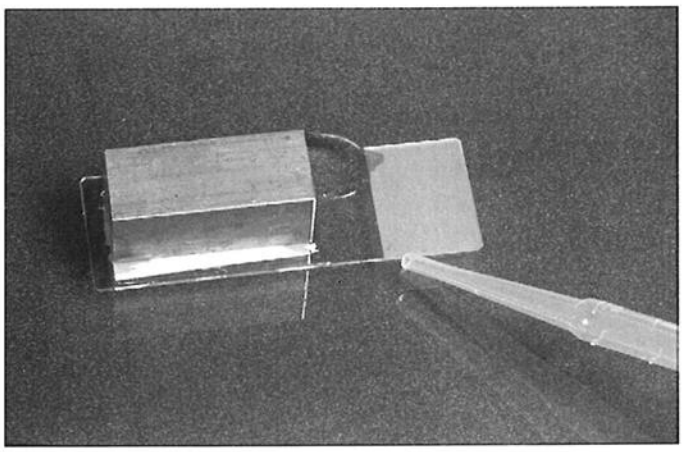

Figure 29: In case the glass slide has got stuck to the thick glass slab, let some xylene flow in between with the help of a pipette.

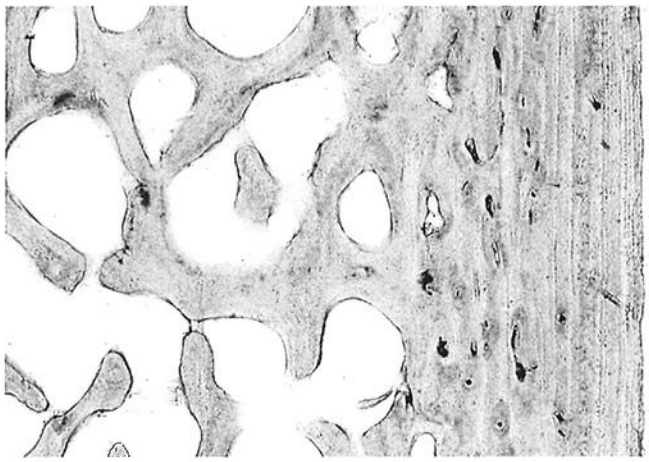

Figure 30: Micrograph of a piece of human parietal bone (external table and diploë) from the Late Mediaeval Period. Section thickness: 11 micron. Bright-field. Microscope magnification 6.6 x.

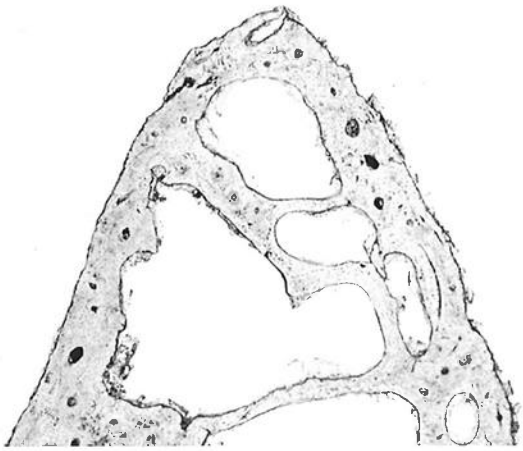

Figure 31: Micrograph of a transverse section of a human rib from the Late Mediaeval Period. Section thickness 12 micron. Bright-field. Microscope magnification 6.6 x.

Figure 32: Detail of Figure 30. Polarized light. Microscope magnification 66 x.

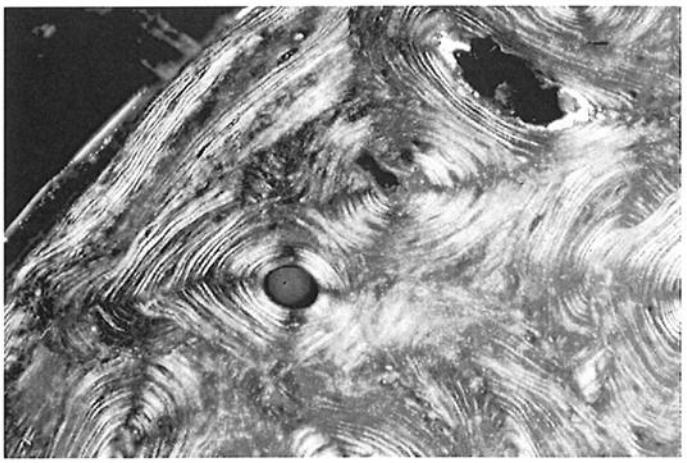

Figure 33: Micrograph of a transverse section through the anterior part of a cremated and heavily calcinated human femoral shaft from the Roman Period. Polarized light. Section thickness 13 micron. Microscope magnification 66 x.

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