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Do it yourself

How to equip a basic histological lab for the anthropological assessment of human bone and teeth

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Introduction

Ancient human skeletal and dental remains are sometimes excavated in fragmentary condition. This extreme condition of skeleton material limits the ability of osteologists to reconstruct the individuals' biological characteristics such as sex, age-at-death, stature, and ancestry. Standard osteological methodologies are often not possible to employ under these conditions. Yet, the desire for a demographic profile remains a specific goal. The employment of standardized histological methods improves the likelihood that unknown skeletal remains will be identified in the forensic context and it provides the bases for the demographic profile required by bio-archaeologists (Kerley, 1965, Stout & Paine, 1992). Other uses for skeletal histology include distinguishing human from non-human bone (Jowsey, 1966; Mulhern & Ubelaker, 2001; Owsley *et al.*, 1985). This is critical if the remains are extremely fragmented and lacking gross identifiable features. A recent use for skeletal histology applies micro-anatomical features (for example secondary osteon, OPD counts, and Osteon area, see Fig. 1a) for determining metabolic problems such as dietary deficiency (Paine & Brenton, 2006a, b). Dental histology is used to assess enamel defects and to determine the timing of their occurrence (Goodman, 1990).

Since there is a growing and much appreciated desire to use the various applications of bone and dental histology for the analysis of human remains there is also an aspiration by numerous researchers with no background in skeletal histology to put this methodology to practice. One of their impediments for doing so is the lack of equipment and the lack of knowledge for developing the laboratory facilities required for conducting this research. The purpose of this communication is to provide the reader with basic information so that the novice researcher can begin to organize a histological lab for these purposes.

The production of thin sections

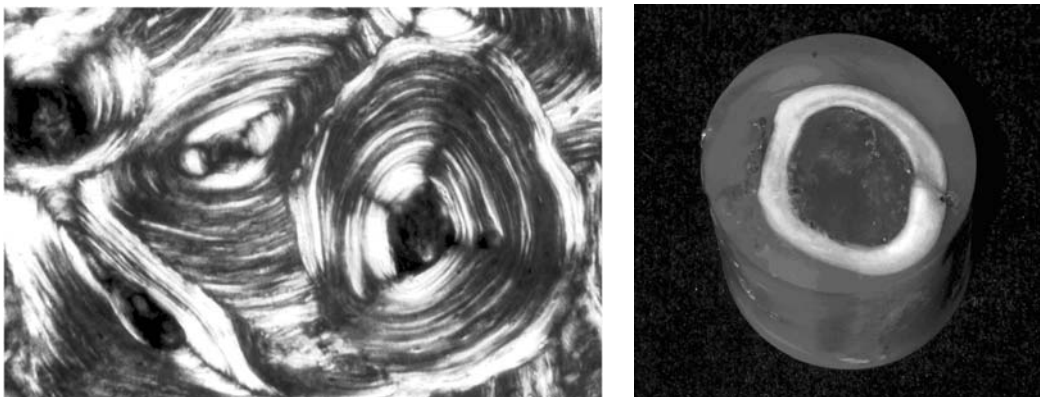
Appropriate histological equipment is critical for the creation of an anthropologically focused histology lab that will be used to product histological slides from dry bone and teeth. A limiting factor to the creation of a histology lab is

the start up cost. The discussion and suggestions provided in this paper will try to offer an easy and cost effective means for creating such a lab.

The preparation of dry bone prior to making bone thin sections used for reading skeletal micro-anatomy is an important first step in this procedure. Maintaining the integrity of the sample during grinding and cutting processes is necessary for accurately documenting the micro-anatomy of both bone and teeth. Therefore, most fragile samples are embedded in an epoxy resin before cutting and grinding. Although, I have found that modern bone (recently defleshed material and boiled bones from forensic cases) with small cross-sections, i.e. rib, clavicle, metacarpal, and macaque femur will not require an embedding step in the preparation of the bone sample for sectioning. On the other hand, archaeological and fragmentary bones will usually require embedding them in a plastic resin before cutting and grinding can take place (Fig. 1b). The plastic resin and hardener EPOTHIN from Buehler, Inc. works very well for this purpose. It takes a day or so for it to air dry and does not require vacuum equipment for the plastic to penetrate the bone. The fact that this resin can be air cured makes it an ideal mixture to work with. There are several ways for forming and enclosing the embedded samples as the resin hardens. The use of clean small plastic boxes or reusable rubber molds is helpful at this stage of the process. These items can be purchased from Buehler.

Once the bone has been prepared for the cutting (that is, it is embedded and/or cut to1-inch lengths) the section of bone is then cut to produce a 1mm thick cross-sectional wafer. To make the wafer cuts, I suggest employing an ISOMET low-speed saw (Fig. 2) with variable size diamond edged blades (4 or 5 inches in diameter) along with several different sized and shaped holding chucks (see Fig. 2) and a swivel arm from Buehler, Inc. I have found this equipment to be the least expensive to use and it does a very fine job. Most critically, the equipment is easy to use and can be mastered by most undergraduate students learning how to make the bone and teeth wafers. Alternative cutting and grinding devices include the PetroThin equipment from Buehler and various Microtomes that can be set up to do both grindings and cutting of bone and teeth automatically. These devices are considerably more expensive to buy and to maintain.

Once the wafer is cut and the edges of the bone or plastic resin are smoothed with sand paper and water to eliminate burrs that can be created by the cutting process; the wafer



a b

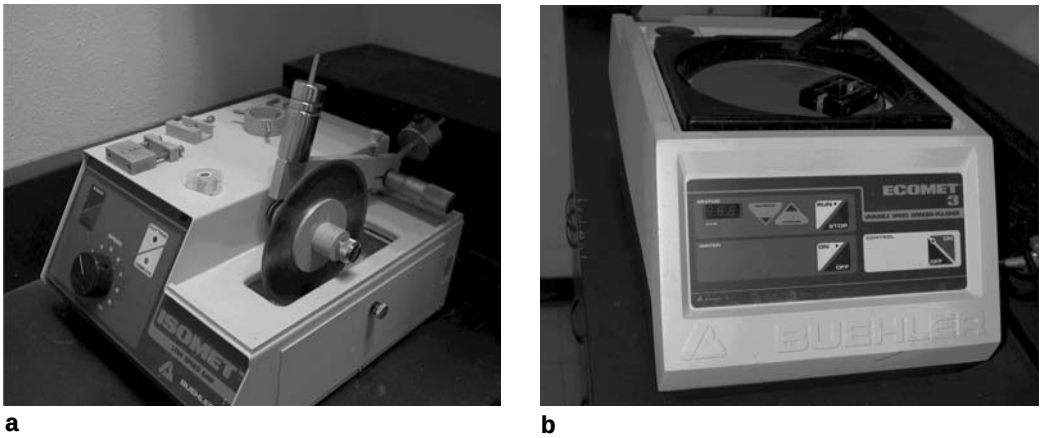
Fig. 1 – a) Secondary osteons from a human section of bone, photographed using polarizing light (for publication purposes the photos was modified to a black and white format); b) 1-inch section of femoral shaft embedded in epoxy resin (the embedding is done to archaeological bones and teeth before the cutting a wafer from the section).

can then be laid perfectly flat on to the frosted grinding slide. The wafer should then be glued to a frosted grinding slide with permount. Permount is the preferred glue of choice simply because it can be dissolved with xylene. One can buy this glue from the Fisher Scientific company. I use plenty of permount to affix the bone to the grinding slide and I like to place a small piece of paper towel on top of the wafer so that I can then clamp the wafer to the slide with a paper clamp. The small piece of paper towel prevents the clamp from attaching to the wafer. The wafer should remain clamped to the slide for 5 days so it can set firmly to the frosted grinding side. If the wafer is not set well, it will detach from the frosted slide during the grinding process.

Once the wafer is firmly attached to the frosted grinding slide, it can be ground down to approximately 75 microns thick using an EcoMet variable speed Grinder/polisher (Fig. 3). I typically use 400 or 600 grit sanding paper for this purpose. The larger the grit number for the sandpaper, the less likely one will product thin sections with a micro-scratched surface. The EcoMet and sandpaper are also from Buehler. The EcoMet grinder requires a water source, which is used to lubricate the sandpaper during the grinding process. Typically, a plastic/rubber tube is used to connect the water supply from the sink to the EcoMet polisher.

Mounting the thin section on to a reading microscope slide is the next step. The thin section should now be approximately 75 microns thin. The thickness of the bone can be determined by eye, it should have a transparent look to it. If the section has an opaque look to it, it may require further polishing. Making the judgment of the section thickness requires some practice before getting it right. After the polishing is finished, the thin section needs to be removed from the frosted grinding slide and cleaned. This can be done by using an ultrasonic cleaner to remove debris from the section of bone while also removing the thin section from the frosted slide. I used xylene in a small glass jar with a cap to clean and remove the section for the grinding slide. Place the jar of xylene in the sonic cleaner; the sonic cleaner is about half-full of water. Once the thin section is removed from the frosted slide, it needs to be very carefully taken from the jar of xylene, air dried and placed on to a reading slide. To place the thin section on to a reading slide use permount and cover the section with an appropriate sized cover-slip. After the glue is set, the slide can be read with a light microscope.

Generally, the process of making a thin section will require a minimum of 6 to 8 days



a b

Fig. 2 – a) IsoMet slow speed saw with a 4-inch diamond edge blade. The bone thin section is embedded in epoxy resin. Several of the chuck holders used to hold the bone in place during cutting are shown on top of the IsoMet; b) EcoMet multi-speed grinder with a plastic slide holder used for grinding the frosted grinding slides (600 grit sandpaper is used to grind thin sections of bone and teeth).

of preparation. This includes the 1-2 days for the embedding to take place, 5 days for gluing the thin section to the frosted grinding slide and allowing the glue to dry. An additional day is required to grind the bone wafer into a thin section and then to have it placed it onto a reading slide. The process can move quickly if you are making several thin sections at a time. If you do decide to make more than one thin section at a time you should label them so they are not mixed up. The frosted grinding slides can be written on with a pencil. The permount and xylene with effect ink so it is wise not to use a pen of any sort to label the glass slides.

The cost of maintaining the equipment is minimal. I have used both the IsoMet saw and the EcoMet grinder/polisher for 14 years with no maintenance cost at all to this equipment. Over time and with use, the diamond blades will require replacement. Otherwise, the only up keep for this part of the lab includes stocking it with grinding paper, frosted grinding slides, reading slides with cover slips, embedding medium, and permount.

Microscopes and the reading of thin sections

Minimal requirement for reading thin sections of bone or teeth include a basic light microscope equipped with a 10x ocular with appropriate grid and variable objectives. For example, one might use an Olympus microscope BX51. To maximize ones potential use and reading easy, I recommend that the microscope be fitted with polarize lighting. The polarizing light helps to define the micro-anatomical features (i.e. secondary osteons) and it provides great lighting for photographic purposes. Minimally, one should have 10x oculars and maybe a second pair of oculars at 20x. Objectives might range from 10x, 20x, 40x, 100x. Additional objectives might include 1.2 and 5x these objectives can be placed in the phototube of the microscope and are used during photographing the cross-section of bone.

Most skeletal histologists are now using some form of image analysis system to collect quantitative data, there are several systems to pick from and these systems are critical for measuring the area of micro-anatomical features (osteons, cortical bone and Haversian canals) but are not necessary for performing Kerley (1965), Kerley & Ubelaker (1978) or Stout and Paine (1992) aging methods. Regardless of the technique or system used one will also need a basic stage micrometer for setting scale while taking area and length measurements (this would be done for each ocular and objective setting).

Digital cameras and computers organized to handle data and images are necessary. Once again, a number of companies offer this type of equipment; I have recently begun to use "Pax-it" digital equipment and recording system. However, I am sure there are other options out there that are just as good. In the past, I have used Bioquant and Optimas imaging systems. What is required of an image analysis system is the ability to capture images on the computer for the purpose of quantifying the histomorphometrics, that is for taking length, area, perimeter and counting data from the cross-section of bone. Unfortunately, I have not found any system that could take these data points automatically; each measure has to be done manually.

Final Remarks and Suggestions

To develop a functioning histological lab for producing and reading thin sections one has to bring together specialized equipment from a variety of sources. Once the lab is set up, research on bone identification (animal vs. human), for measuring micro-anatomy of hominid fossil fragments, age assessment, health assessment, for biomechanical analysis of cortical cross sectional area and for the examination of dental defects can be done. The purpose of this article is to provide the reader with direction and suggestions for getting started as they organized their own skeletal-dental histology lab. Figures 2-3 show some of this equipment and Appendix 1 offers a list of equipment, approximate cost as of 2007, and the manufactures of the commonly used items. This information should

help to organize the reader as they begin this process. Specifically one could put together a functioning histology lab for around $30-75,000.

A last word of advice, in the end, the results obtained from the analysis of skeletal and dental remains via histological methods are only as good as the training one has. Accurate measuring of micro-anatomical features can only be accomplished with the guidance of an established histologist; one who has worked with histological features of bone and teeth for a considerable period. The training of a skeletal/dental histologist cannot occur simply by reading literature; the knowledge and skill must be obtained from individual instruction. This last observation may be the most limiting factor to the degree of success a researcher has as they begin to explore the potential that skeletal histology as to offer to the physical anthropologist as they record histomorphometric data from bone and teeth.

Info on the web

- http//www.buehler.com/productinfo/gp1.htm
  Cutting and grinding equipment, and thin sectioning supplies.

- https//www1.fishersci.com/index.jsp
  The disturbers of Permount.

- http//www.leica-microsystems.com
- http//www.olympusmicro.com/
  Suppliers of light microscopes.

- http//www.reticles.com/countingreticles.htm
  Stage micrometers and counting reticules.
- http//www.paxit.com/
  Image analysis systems with digital cameras.

- http//www.mediacy.com/index.
  Optimas and other systems.

- http//www.bioquant.com/
  Image analysis systems with cameras.

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Appendix 1 - A list of basic histology items, manufactures, and an estimation of cost of the equipment.

Item	Description	Size	Estimated cost (USD)
ISOMET low-speed gravity saw	A cutting device for making thin sections	26 x 29 cm	4,000
Image analysis systems with camera	Used for collecting quantitative data		5,000-20,000
UltaMet sonic cleaner	For cleaning slides and bone wafers	10 x 10cm	100
EcoMet 3000 & 4000	For grinding the thin sections	66 X 32 cm, requires a sink and water tap.	4,000
Light microscope and variable objectives	For reading the slides	152 x 76 cm work table for microscope and computer system	15,000-20,000
EPOTHIN	Plastic resin and hardener	Resin 0.95 liter, Hardener 0.47 liter	67, 36
Diamond blades for IsoMet 4" & 5"	For use with the low speed saw	4-5 inches diameter	255-375
Permount	Mounting medium	N\A 100 ml	21