

# ARE YOU SURE IT'S BONE?

## AN EXPERIENTIAL LEARNING COLLECTION FOR THE HISTOLOGICAL COMPARISON OF BONE AND OTHER MATERIALS

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### Introduction

Bone is a complex material with species-specific microstructures. Despite its unique properties, human bone may be confused with other faunal bone, synthetic, or organic materials, especially to the untrained eye and when fragmented. Studies suggest that between 25 % up to 90% of bone brought to forensic anthropologists consists of non-human bone (Bass, 1995; Donlon et al., 2020).

#### The aim of this project was to:

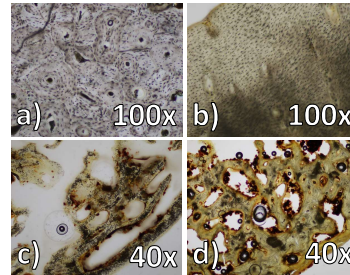
- Develop a histological teaching collection of materials
- Have the collection be accessible at the BAFAL Lab
- Permit training for students, volunteers, and local law enforcement personnel in the identification of different materials
- Open opportunities for further undergraduate research

### Methods

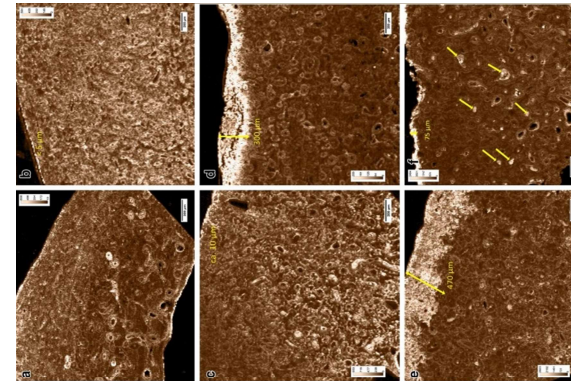
SRAP (Student Research Assistant Program) students and volunteers collected a variety of samples for this project based on macroscopic or expected microscopic structural similarities to bone. Examples include coconut shell, a skull-shaped jewelry bead, an eraser, a stick, and a soft pretzel. Each sample was cut into a 1 cm segment. Epoxy resin was mixed in a 2:1 ratio of resin to hardener. Samples were then placed in small cups and resin was poured over them and allowed to cure for at least 24 hours. Once set, samples were thin-sectioned using a Buehler IsoMet low speed saw using speed 5. Upon thin-sectioning, samples were adhered to microscope slides using epoxy resin. Samples were hand sanded, starting at 200 grit and increasing to 2000 grit sandpaper until at the thickness of 50-100 microns. They were polished and cover slips added using epoxy resin. An Olympus microscope was employed for light microscopy. Images were taken using CellSens software. Magnification is indicated in the bottom right corner of each image.

### Discussion

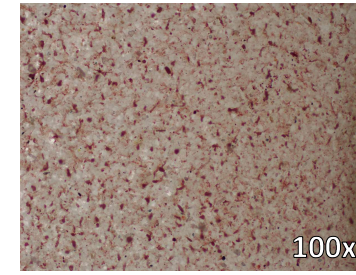
In forensics and bioarchaeology, macroscopic examination could reach its limitation, and microscopy may be employed to more reliably differentiate human bone from other materials as well as non-human bone. To make such assessments, it is crucial to be very familiar with species-specific microstructures and non-bone materials, both macroscopically and microscopically. This collection will educate students, volunteers, and local law enforcement of material microstructures, both at the lab and from a distance. To make the collection fully accessible for training modules and classes taught at BAFAL, the next steps are to digitally catalogue the samples for remote access. Finally, it allows new experiential learning and research opportunities to students at BAFAL.



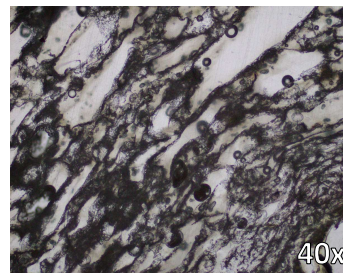
**Figure 1(a-d).** a) Secondary osteonal bone in an adult human femur. Osteons are circular lamellar structures that build a complex network through continuous remodeling and branching. This interconnectivity may have an "overlapping" appearance in cross-sections. b) Primary lamellar bone in a subadult human femur; as visible in the left upper corner of the image, is aligned with bone surface curvature, as it develops in layers. c) Osteoporotic changes in vertebral bone; due to disease or severe osteoporosis. The large spaces may be confused with resorption spaces. d) Amputated femur featuring loss of bone mass and enlarged resorption spaces within compact bone.



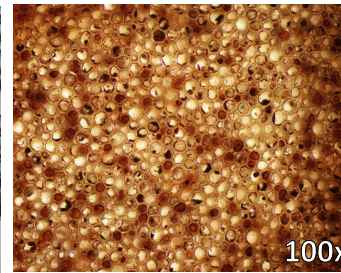
**Figure 2.** Archaeological bone with signs of diagenesis – the collagen is beginning to break down and mineralize the bone (Rasmussen et al. 2019). Images were taken using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). Scale is 200 microns. Degeneration of collagen results in a loss of the lamellar structure typical in human bone, making it more difficult to differentiate from other materials.



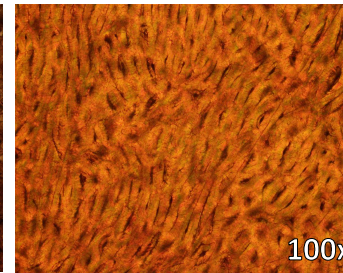
**Figure 3.** The eraser sample may be confused with the fast-growing woven bone seen in subadults and during fracture repair. Secondly, it has similar features to bone that has undergone diagenesis and lost much of its collagen. An example of diagenetic bone can be seen in Figure 2. The difference is that the eraser sample lacks directionality of fibers and does not show remnants of osteons or resorption bays like human bone does.



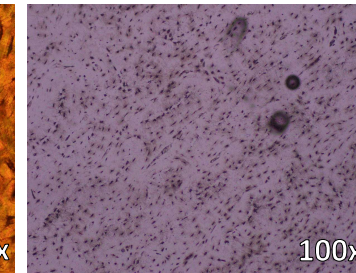
**Figure 4.** The soft pretzel exhibits large pockets of space that align in a uniform direction. This is similar to how strongly trabeculated bone looks, due to disease or severe osteoporosis. The large spaces may be confused with resorption spaces.



**Figure 5.** Central pith of a stick. It has many open pore spaces which may, to the untrained observer, be confused with osteoporosis or diagenetic human bone, but it lacks the lamellar structure of osteonal bone. Bundles of tissue in the stick are all uniform and don't have resorption.



**Figure 6.** The coconut shell features a similar "folding" and layering pattern to that of more youthful bone, with spots that may be confused with osteons or resorption bays. However, the coconut shell does not exhibit regular spaces for fluid canals or regular strength-bearing structure i.e. osteons. It more closely resembles fast-growing faunal plexiform (scaffolded) bone.



**Figure 7.** This sample is from a skull-shaped jewelry charm. Macroscopically, it has characteristics of bone in color, texture, and hardness. We examined it microscopically, and it did turn out to be bone. It has signature regions with vascular canals and osteocyte lacunae but lacks the density of osteons expected in human bone. This bone is faunal, most likely a fast-growing animal with thick cortical bone allowing beads to be carved from it.

### References

- Bass, W. M. (1995). *Human Osteology: A Laboratory and Field Manual*. 4th ed. Columbia, MO: Missouri Archaeological Society.
- Donlon, D., Croker, S., & Menzies, J. (2020). Non-human bones in forensic casework: Not such a trivial problem. *Forensic Science, Medicine, and Pathology*, 16(3), 442–449. <https://doi.org/10.1007/s12024-020-00257-w>
- Rasmussen, K. L., Milner, G., Skytte, L., Lynnerup, N., Thomsen, J. L., & Boldsen, J. L. (2019). Mapping diagenesis in archaeological human bones. *Heritage Science*, 7(1), 1–24. <https://doi.org/10.1186/s40494-019-0285-7>



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