



## Physical Anthropology Section - 2013

### H63 Use of Silver Nitrate Stain to Visualize Microanatomical Features in Decalcified Bone Cross-Sections

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After attending the presentation, the attendees will learn about slide preparation modifications that can be used for visual microanatomical structures on decalcified bone cross-sections.

This presentation will impact the forensic science community by providing a technique for forensic anthropologists to continue their analyses using bone histology without the need for specialized equipment. Additionally, these slides can be prepared within the histology laboratories employed by medicolegal offices.

The use of histological techniques to assess bone is well established in anthropology. It is especially valuable in the applied field of forensic anthropology for the differentiation of human from non-human bone, age estimation, differential diagnosis of bone pathological conditions, and assessment of overall bone health. The standard petrographic technique for producing thin sections typically used by anthropologists involves undecalcified bone samples that are vacuum embedded in molds filled with epoxy resin and hardener. Once hardened, 1mm sections are cut from the embedded sample block using a diamond-blade saw and ground to a thickness of approximately 70µm. Due to the expense of specialized equipment and supplies, the petrographic technique is not generally available at medicolegal offices. The standard bone histological technique used in medicolegal offices to produce thin sections involves cutting decalcified bone into sections approximately 4µm thick using a microtome and staining with hematoxylin and eosin. Unlike petrographic sections, standard histological sections of decalcified bone are too thin for visualization of cement lines, a key feature used by forensic anthropologists to identify osteons and fragments. Previous studies have shown that silver nitrate stains can successfully be used to demarcate cement lines. Since silver nitrate adheres to calcium and darkly stains calcified tissue, the contrasting unmineralized cement lines remain unstained. This study explores the use of silver nitrate-stained decalcified sections in histomorphometric analyses of bone for the purposes of age estimation.

Samples from the anterior third of the sixth rib were collected from unidentified decedents who were exhumed as part of the Harris County Institute of Forensic Sciences Unidentified Decedent Review Project. Sections were prepared using the standard histological procedure where the samples were formalin-fixed and decalcified prior to embedding in paraffin wax. Once set, 8µm slices were sectioned from the paraffin block and placed on a slide. Eight micron sections were selected because they are thick enough to provide sufficient contrast between bone tissue and cement lines without causing the section to lift off the slide, as can happen with thicker decalcified bone sections. The sections were treated using a silver nitrate stain and cover-slipped. Once the slides were prepared, age was estimated for each sample following the Stout and Paine method as well as Cho et al. method using anterior cross-sections of the sixth rib.<sup>1,2</sup> These estimates were compared to the age estimated for the decedent using macroscopic techniques, in particular the sternal rib method and the pubic symphysis method.<sup>3,4</sup>

Histological age estimates derived from the decalcified sections, particularly using the Stout and Paine method, were within or close to the macroscopic age estimates for the decedents in the sample population.<sup>1</sup> The histological point estimate of age obtained for each rib was compared to the phase mean assessed for the pubic symphysis and/or sternal rib methods. Although there were differences in estimates between the microscopic and macroscopic groups, no consistent pattern was observed. The histological point estimate of age either fell within the 95% confidence interval for the macroscopic methods or exceeded the interval, therefore producing an overestimate. Since there is no clear understanding of how histological age estimates derived from petrographic slides compare to the macroscopic techniques, the efficacy of the decalcified method compared to the petrographic method could not be evaluated. However, because the age estimates are relatively close, these findings suggest that the decalcified silver-stained sections are appropriate for use in histological age estimation and the method should be explored further.

A limitation of the method is the use of extremely thin bone sections that have a tendency to fold when mounted on a slide, thus reducing the amount of bone that can be read. Tears and obliteration of the periosteal surface was also noted in some slides.

#### References:

1. Stout S, Paine R. Brief communication: histological age estimation using rib and clavicle. *Am J Phys Anthropol* 1992;87(1):111-5.
2. Cho H, Stout S, Bishop T. Cortical bone remodeling rates in a sample of African American and European American descent groups from the American Midwest: comparisons of age and sex in ribs. *Am J Phys Anthropol* 2006;130:214-26.
3. Iscan M, Loth S, Wright R. Metamorphosis at the sternal rib end: a new method to estimate age at death in White males. *Am J Phys Anthropol* 1984;65:147-56.
4. Brooks S, Suchey J. Skeletal age determination based on the os pubis: a comparison of the Acsadi-Nemeskeri and Suckey-Brooks methods. *Human Evolution* 1990;5(3):227-38.



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**Bone Histology, Decalcified Bone, Stain**