



## Physical Anthropology Section – 2005

### H73 Measure Twice, Cut Once? Measurement Error Levels in Histomorphometry

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After attending this presentation, attendees will understand a method to assess repeatability between measurements, the amount of measurement error within rib cross-sections and between serial cross-sections, and if multiple cross-sections are warranted for evaluation.

This presentation will impact the forensic community and/or humanity by underlining the importance of exploring measurement and sampling error levels in histological methods. This research stresses that the repeatability of measurements is paramount and demonstrates a statistical method to evaluate measurement agreement. Addressing issues surrounding repeatability are the first steps to further the use of quantitative bone histology in the field of forensic anthropology.

Compared to the myriad of gross morphological methods available to forensic anthropologists, histological methods for estimating age at death are underutilized. Uncertainties regarding how much bone to evaluate and the associated levels of sampling error may cause this underutilization. Significant sampling error can occur within and between cross-sections, with the latter's sampling error increasing dramatically as the amount of cortical area evaluated decreases<sup>(2)</sup>. A rib cross-section with 10mm<sup>2</sup> of cortical area may demonstrate several hundred percent difference in bone formation between serial bone cross-sections<sup>(5)</sup>. Frost<sup>(2)</sup> recommends a minimum of 50mm<sup>2</sup> of cross-sectional bone in non-pathological individuals be analyzed to minimize sampling error. Stout<sup>(4)</sup> asserts that the time necessary to increase the amount of histological samples must be weighted against the gains. It is for this reason that he recommends the evaluation of two rib cross-sections per individual. Conflicting reports of measurement error within and between studies may arise due to inadequate methods of error assessment, such as correlation or paired t-tests, producing misleading results<sup>(1)</sup>. Another issue is the absence of clear guidelines for how much error is acceptable. This ultimately means that it is the decision of the researcher; however, a 10% error level is commonly used. The goals of this research are to evaluate precision in rib intra-site histomorphometric measurements and assess inter-site measurement agreement to determine if two cross-sections are adequate.

Two rib cross-sections were evaluated from 30 individuals of known age and sex (14 females, 16 males; aged 27-79 yrs) selected at random from a collection of 234 individuals from the cemetery site of Spitalfields, London. None of the individuals demonstrate any gross or histological morphology indicating a pathological condition. Osteon population densities (OPD = number of intact + fragmentary osteons ÷ surface area) were recorded following the Stout (1986) method. A plot of the difference between measurements against the mean of the first and the second measurement was utilized for both intra and inter-site values. Absolute mean percent differences were calculated to quantify the magnitude in variability between measurements with the 10% error level as the cutoff for acceptance.

The results show that absolute mean percent difference in OPD values for the intra-site evaluation is 8.5% indicating repeatable measurements. The mean difference is not significantly different from zero, further indicating that repeatability was achieved. The absolute mean percent difference in OPD values for the evaluation of serial cross-sections is 15%. The plot of the mean difference indicates a magnitude bias; therefore, the data was logged transformed to provide a clearer picture of agreement. The overall mean difference was significantly different from zero indicating that repeatability was not achieved. Interestingly, the minimum difference in error between serial sections was less than 1% and the maximum was ~ 50%.

Overall intra-site precision error is below the 10% level, but individually 7 of the 30 samples exceeded the acceptable error level. Fifteen of the 30 samples demonstrate moderate to large amounts of diagenesis. Six of the 7 samples that failed acceptance are within the diagenetic group. When diagenesis is present a cross-section should be measured twice and the average value recorded in order to minimize the chance of intra-site measurement error.

Overall the inter-site error for the serial cross-sections failed to meet the 10% level of acceptance, but individually 12 out of the 30 were below the 10% level. These samples have a mean cortical area of 16.5mm<sup>2</sup> (range: 9-24mm<sup>2</sup>), while the rejected samples have a mean cortical area of 13.8mm<sup>2</sup> (range: 8-20mm<sup>2</sup>). The amount of cortical area overlap and the lack of a pattern between error levels and diagenesis, indicates that serial section measurement variability is random. A slight magnitude bias in the error was detected, indicating a systematic increase in error as OPD increases. Because OPD is a calculated value, it is difficult to determine the cause. Most likely, it is a combination of the decrease in



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cortical area and the increase in fragmentary osteons over time. Because substantial differences can exist between serial cross-sections, measuring one section twice will not account for this error. This indicates that the evaluation of multiple cross-sections is required. As a result of the random nature in measurement error (despite the slight trend with increased error and decreased cortical area), Stout's recommendation for the use of only two rib cross-sections from a normal individual should be sufficient in the attempt of minimizing the potential error.

### References:

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3. Stout SD. The use of bone histomorphology in skeletal identification: The case of Francisco Pizarro. *J Forensic Sci*, 1986; 31(1):296–300.
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### Precision, Histomorphometry, Osteon Population Density