Recognizing and Resolving Inconsistencies and Inaccuracies in Determining Osteon Circularity: Can Methods be Standardized?

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Introduction

Osteon circularity (On.Cr) might help in distinguishing species, interpreting load history, and estimating age. When studying On.Cr using our archived backscattered electron (BSE) and circularly polarized images of various primate and non-primate bones, we recognized that inaccuracies can occur when there are seemingly inconsequential differences in scale of actual/physical images used in osteon tracing (although all taken at 50-62.5x). For example, errors might occur if On.Cr data from non-digitized Polaroid images (500microns=26mm) from 1980s-1990s are compared to On.Cr data from modern digitized images in larger format (500microns=53mm). How close must image 'sizes' be to achieve <2% error (arbitrary cutoff)? Additionally, do manual/semi-automated computer-mouse-based tracing provide similar accuracy, and how do these compare with tracing using pen-on-plastic transparencies vs. a digitizing tablet/stylus?

Additional issues considered in this study included addressing these questions (using modern (recent) digitized images): (1) Must crenulations (Howship's lacunae) be traced to achieve On.Cr accuracy?, (2) What electronic tracing method/tool is best?, and (3) Is the use of a stylus and tablet necessary? (This latter one is still under investigation).

Methods

For the first experiment ten osteons (50x BSE images; human femur, 60 years) were manually pen-traced on transparency film and scanned for analysis in ImageJ. Quantifying On.Cr this way is highly accurate when using "Image 1" (Skedros 2000, J.Bone & Mineral Research). The image was then reduced ~25% (500micron=39mm) and ~50% (500micron=26mm) of its original size to resemble our archived/unadjusted images, and the osteons were manually re-traced/re-scanned. Differences ("errors" vs. 100% size) included: (1)25% reduction 2.1±2.5% (max 7.5%), and (2)50% reduction 3.1±2.5% (max 6.0%). Additional analysis showed <2% error when reduction is <15%.

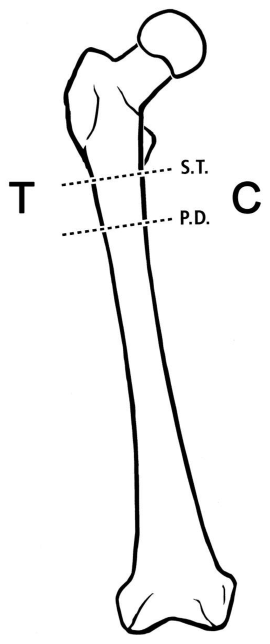
The additional questions were addressed using digital BSE images from middle-aged human femora. The additional findings are shown graphically at near right, with statistical analysis at far right.

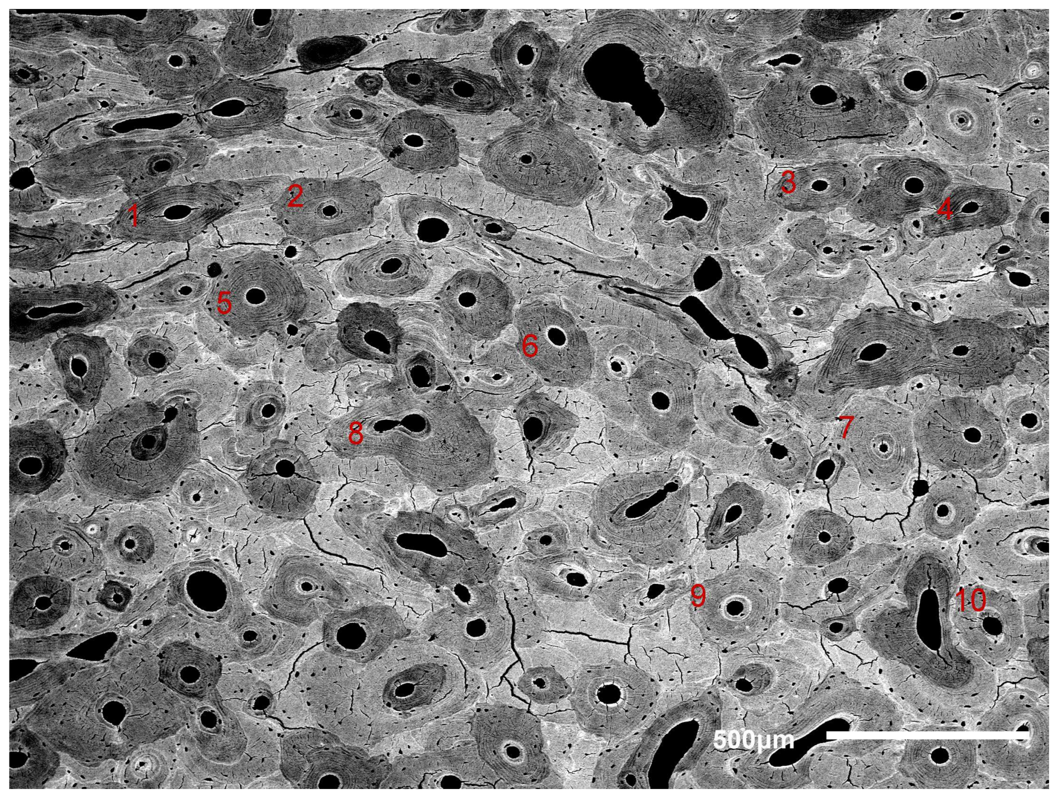
Percent Difference of Osteon Circularity When Compared to Original Size

	Mean	Standard Dev.
25% Reduction	2.1%	2.5%
50% Reduction	3.1%	2.5%

Methods and Results

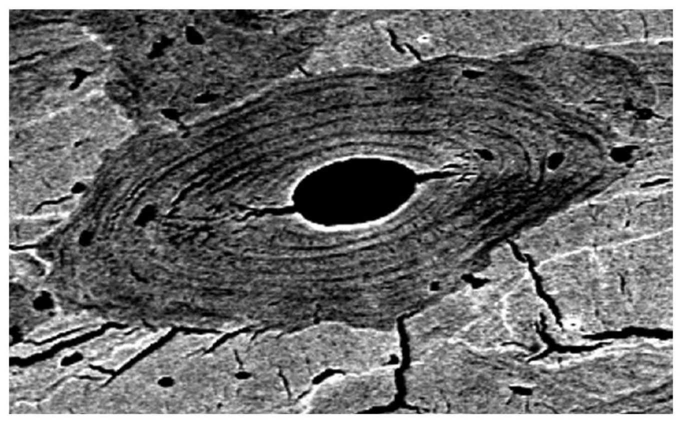
The same ten osteons were traced using the semi-automated 'Quick Selection Tool' in Photoshop [to outline and paint the osteons] and the new image was opened in ImageJ for analysis. Analysis in ImageJ included the 'Wand (tracing) Tool' [to trace the painted osteons] and the 'Fit Spline' function [to smooth the tracing for accurate measurement]. The 'Fit Spline' method was the most accurate with an average error of 1.5±2.3 (max 6.1%). These same traces analyzed in ImageJ using the 'Wand (tracing) Tool' had the most error (19.0±4.3%). The 'Polygon Selections' tool was also used to measure these osteons. We found that when at least 20 points are used, the On.Cr. measurements were similar.

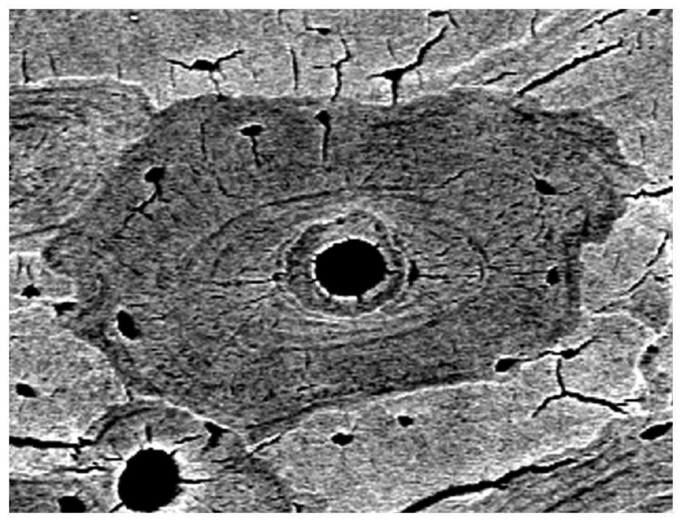




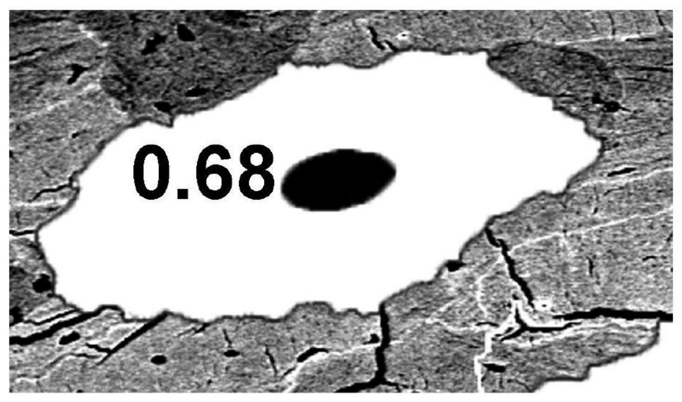
Analysis of On.Cr With and Without Crenulations

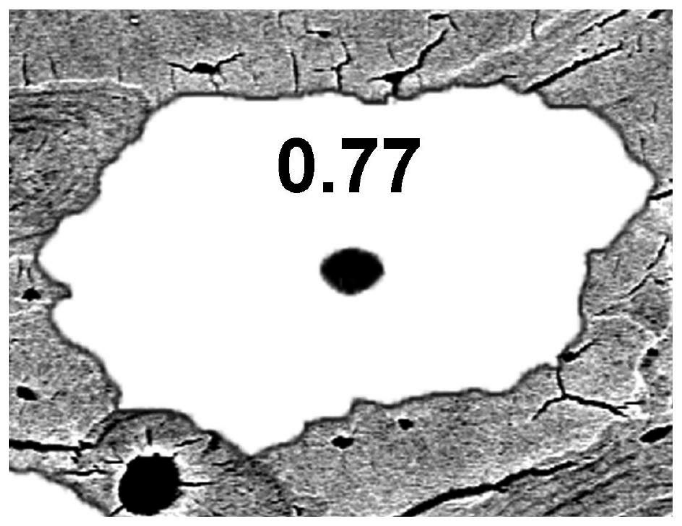
Original Osteons



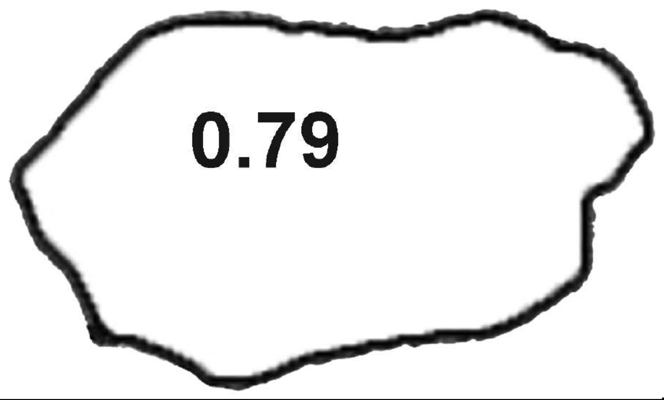


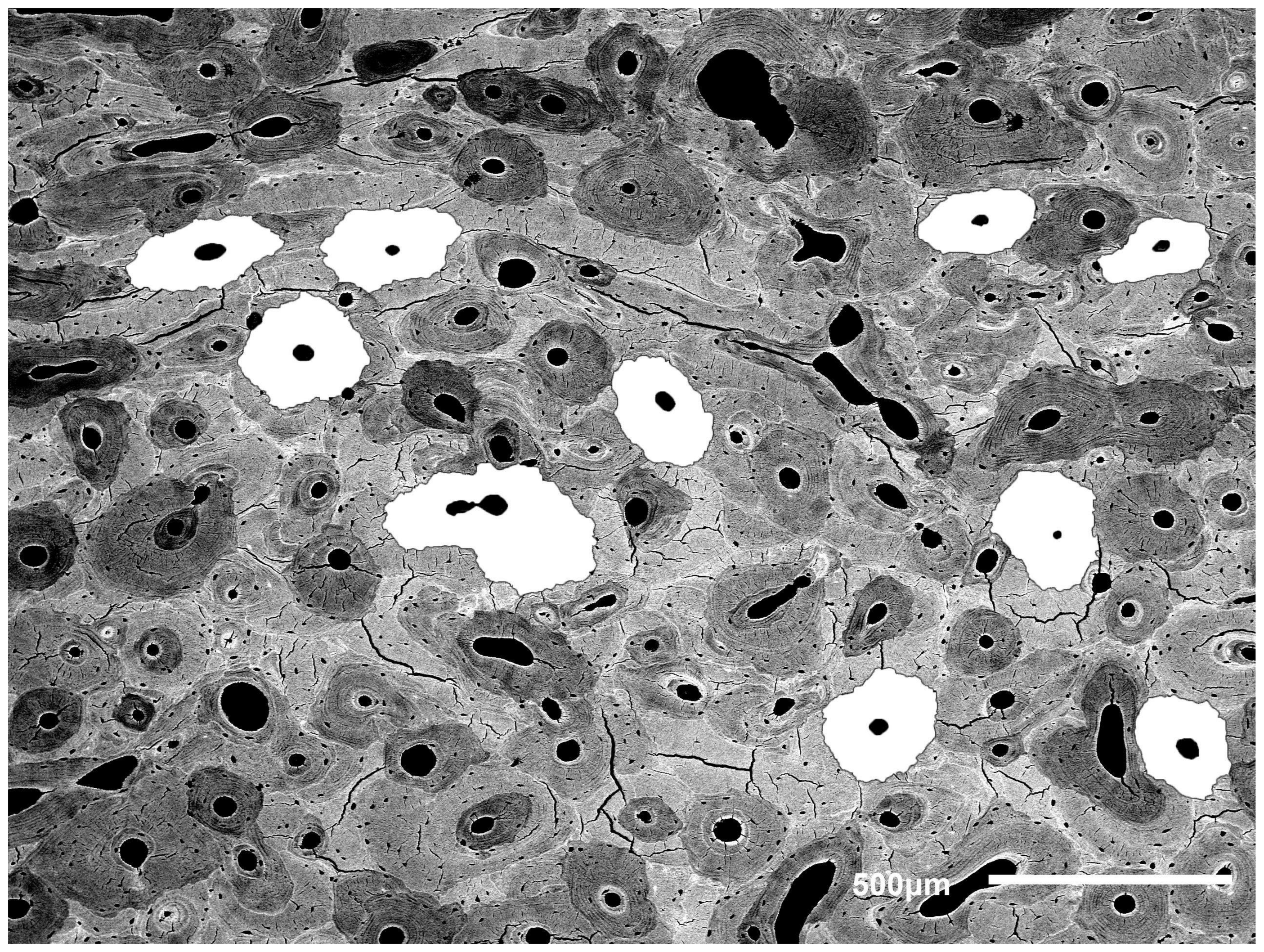
Computer-based Tracings Using Photoshop Quick Selection Tool

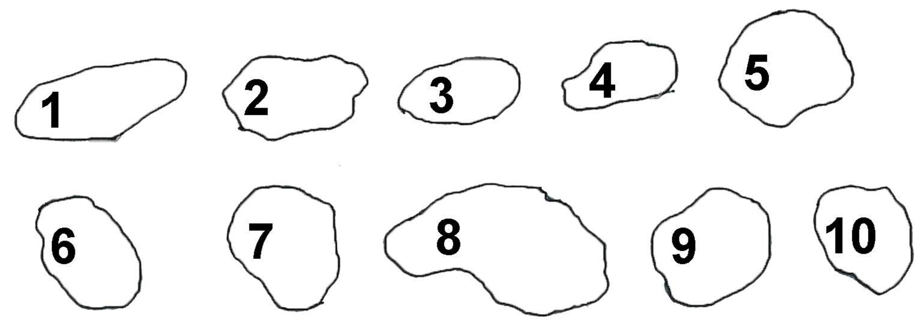




Scanned Hand Tracings







Percent Difference of Osteon Circularity: Smooth Traces vs. Crenulated*

Mean	Standard Dev.
-4.57%	2.96%

*A T-test revealed that the osteon circularity was not statistically different (p = 0.4).

Discussion

The data show that small-scale non-digitized archived images should be avoided in studies of On.Cr.

Tracing crenulations is not necessary for accurate On.Cr. Measurement. This is encouraging for On.Cr analyses that use light microscopy images from sections that are typically much thicker (100µm)

Achieving accuracy when quantifying scanned pen-tracings in ImageJ can be challenging — as especially shown when using computer-generated perfect circles.

Additional studies will be conducted to determine if a digitizing tablet and stylus, as used by Crescimanno and Stout (2012), increases accuracy and efficiency.

References

Crescimanno A, and Stout SD. (2012) Differentiating fragmented human and nonhuman long bone using osteon circularity. J Forensic Sci 57:287-294.

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