Improving Accuracy, Precision, and Efficiency in Analysis of Osteon Cross-sectional Shape

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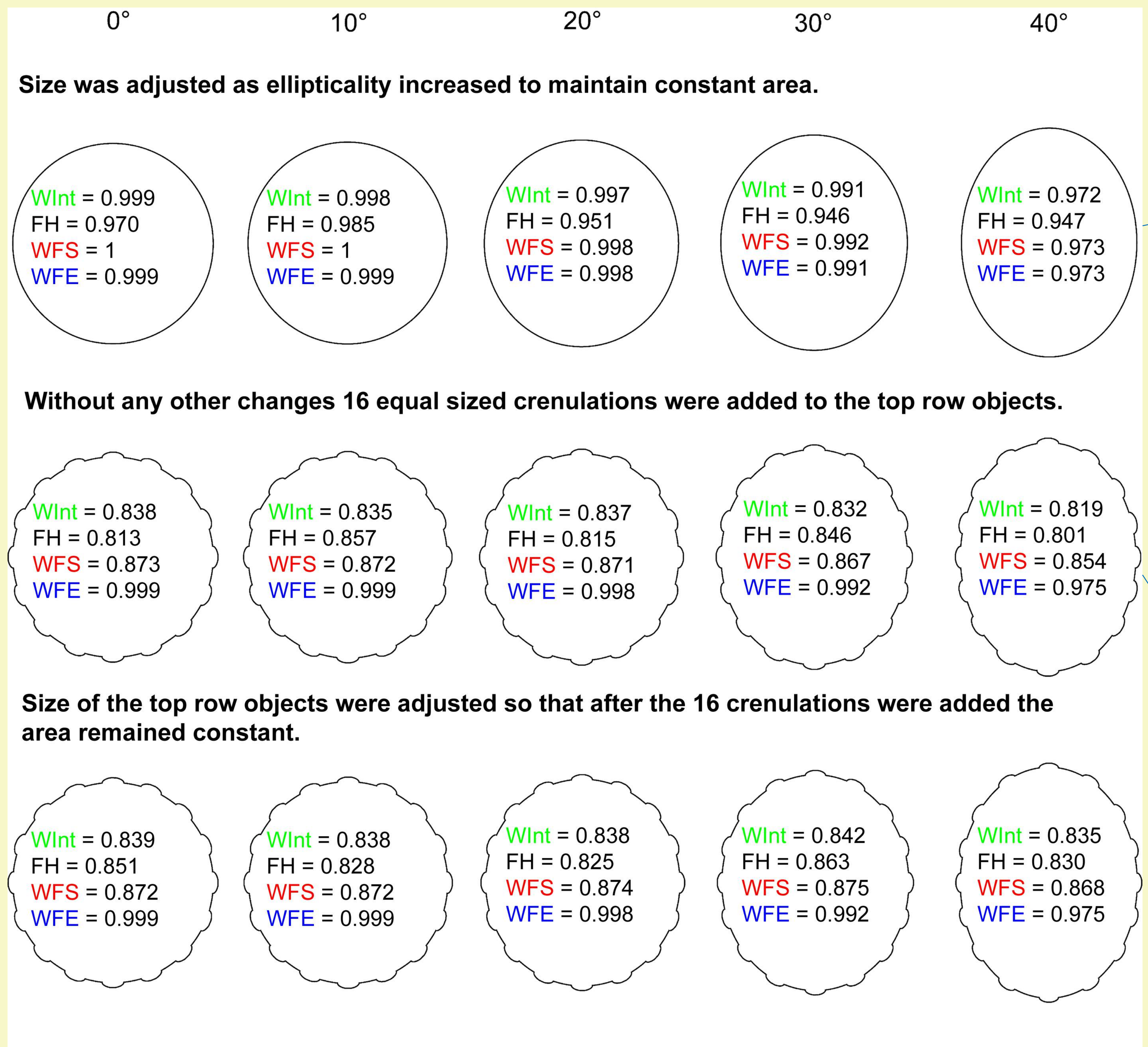
Introduction

Variations in secondary osteon cross-sectional shapes help determine species affiliations, estimate age, and decipher load history [1,2]. Secondary osteon cross-sectional shape is expressed as "circularity index" [CI=4π(area/perimeter²); 1.0 = perfect circle] and can be measured in various ways. But which method is the most accurate and efficient? Depending on the method used, circularity values can be misleading as well as hard to replicate. Studies that have been done on the same secondary osteon have provided circularity values (CI = On.Cr) that differ by 10-20% [3]. This can lead to confusion and misinterpretations.

Methods

Sixty secondary osteons (5 osteons/image; 8 adult deer calcanei and 4 adult human femora; backscattered electron images mostly) and several sets of virtual osteons (created using Adobe Illustrator) and were traced/measured (in Adobe Photoshop). For the deer calcanei and human femora each trace followed a predetermined cement line made by: (1) opening each image in Photoshop, (2) selecting the osteon using the quick select tool, and (3) outlining the secondary osteon periphery with black and filling with white. Using ImageJ, methods included: (1) wand tracing tool followed by smoothing (fit spline vs. interpolate), (2) manual tracing with stylus on a Windows-based tablet (ASUS M80T) using freehand selections tool, (3) manual tracing with the polygon selections tool (minimum 20 points), and (4) the fit ellipse tool. **Secondary osteon area, perimeter and shape were evaluated. Circularly polarized light images were also used in some cases.**

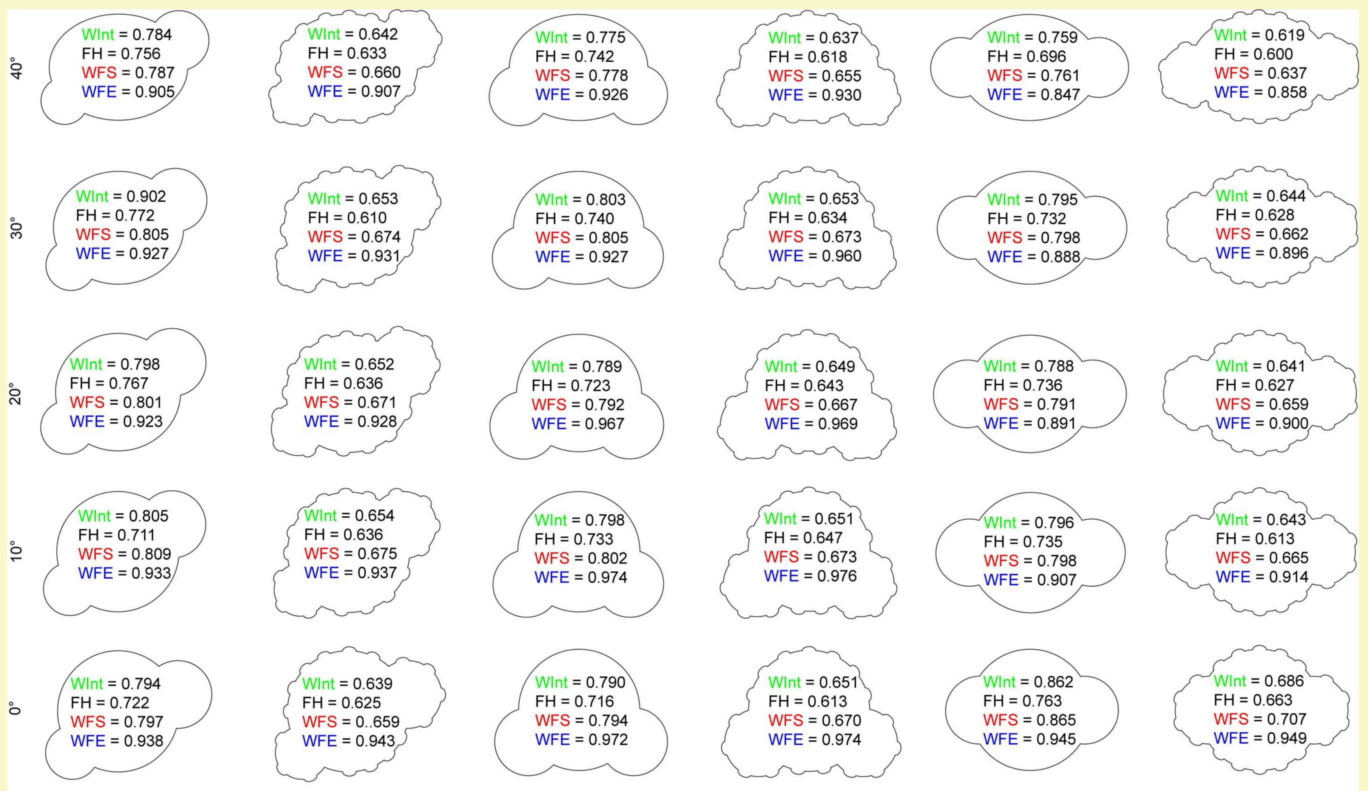


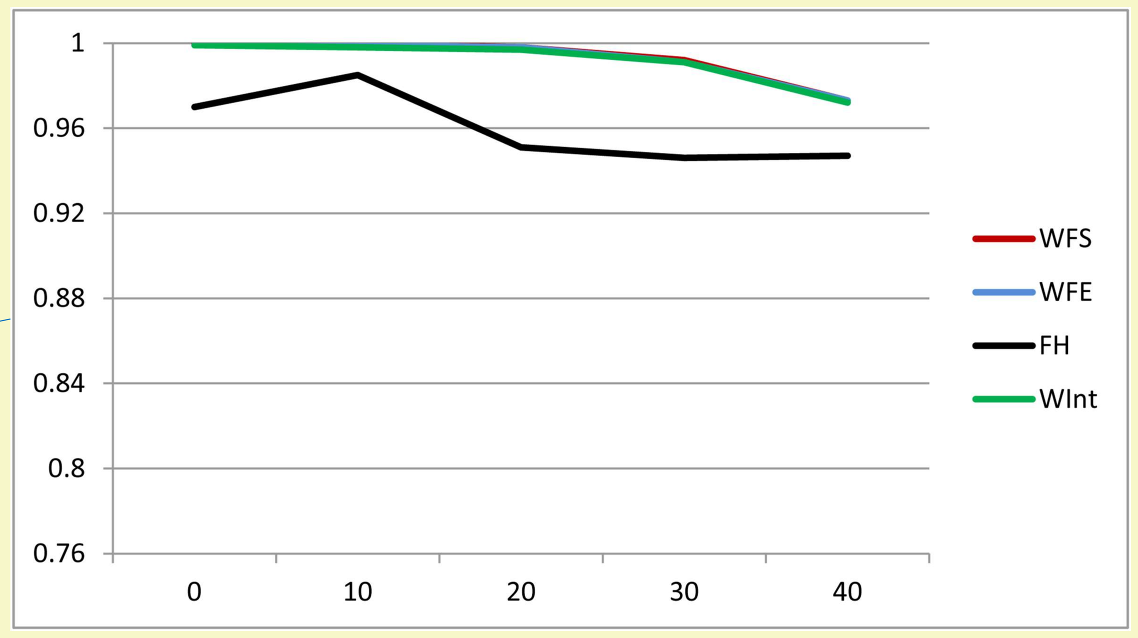
WInt = Wand tool followed by the fit interpolate function.
FH = Freehand tool using a Windows based tablet.
WFS = Wand tool followed by the fit spline function.
WFE = Wand tool followed by the fit ellipse function.

Methods and Results

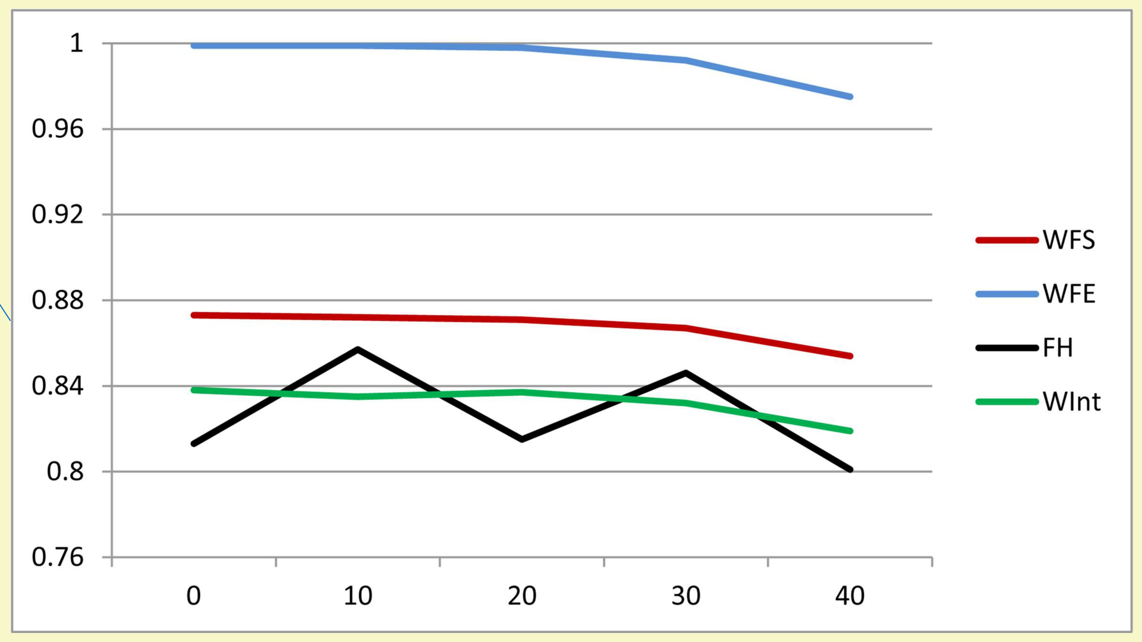
Data from virtual osteons suggest that fit spline could be problematic by over smoothing when crenulations are present, although not by much (mean On.Cr difference = 0.015 vs. interpolate). Although when using the wand tool, fit spline or interpolation can significantly affect perimeter (hence On.Cr); but secondary osteon area measurements are not significantly affected. The greatest errors occurred when using the fit ellipse tool.

Errors were highly dependent on the ability to discern details of the cement line in the images and the ability to trace (freehand). Hence, circular polarized light images were less influenced because crenulations (Howship's lacunae) can not be seen well enough to reliably trace. This contrasts with the BSE images where the contours of the cement line can be seen well.

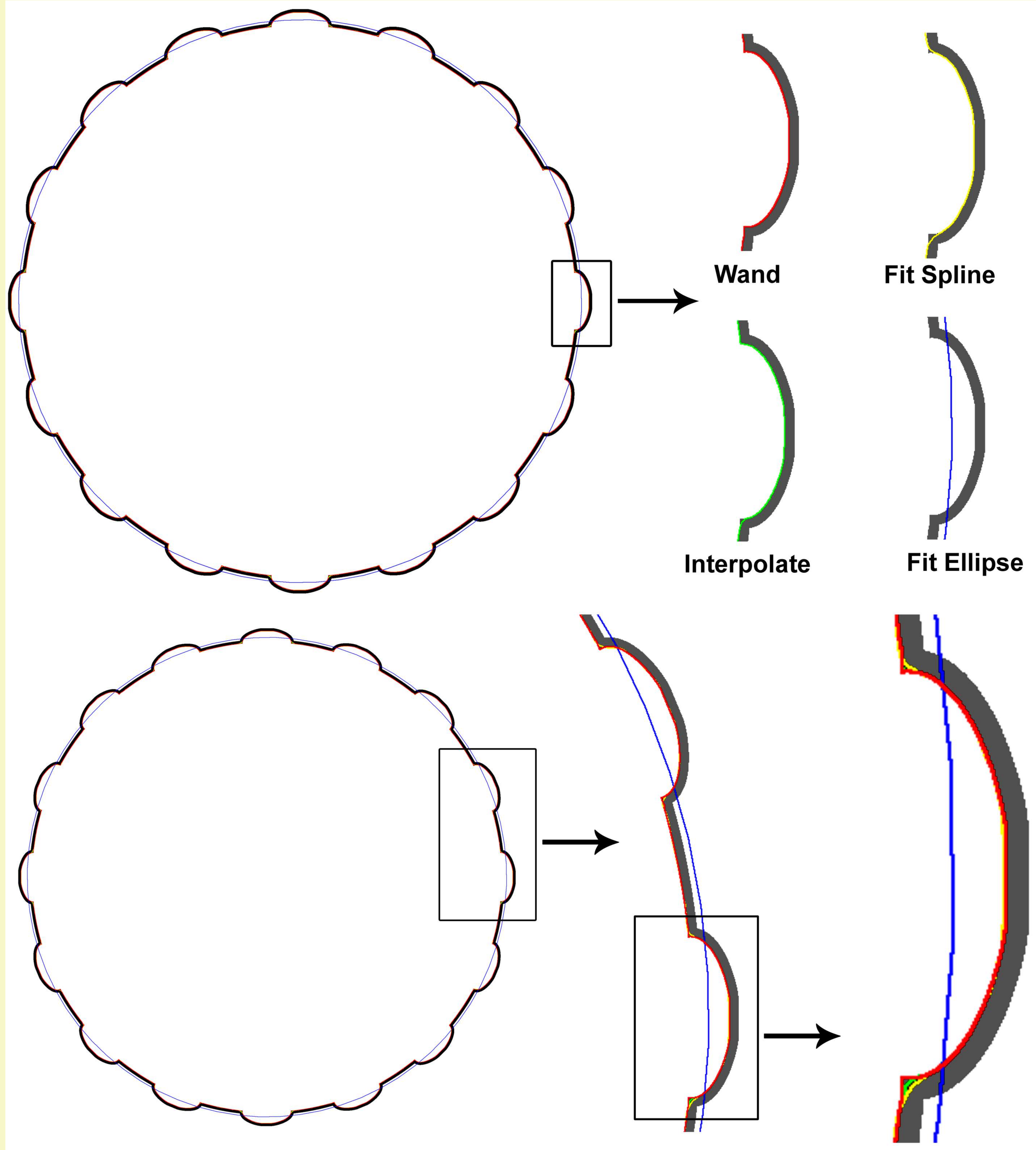




The above graph demonstrates the change in On.Cr with tilt (0˚-40˚) and method. Using the data in the top row of the figure to the left. In this case three methods produced almost identical data (WFS, WFE, and WInt).



This graph uses the data from the middle row of the figure to the left. Notice the difference in On.Cr between methods now that crenulations are present.



Discussion

The best method is the wand tracing tool with interpolation function (WInt). Tracing using a Windows-based tablet running ImageJ/freehand tool, without the fit spline or interpolation functions (neither of which significantly alter the trace) can also be effective. Mean On.Cr difference of these two methods = 0.028. A Windows-based tablet also bypasses the need to first paint the secondary osteon in Photoshop which saves time. However freehand traces can be difficult and will vary with the tracers. The wand tool requires more time but is more accurate and reliable.

The fit ellipse tool was found to be unacceptable when measuring area, perimeter, and/or circularity, because of the high errors in our images. This method would only be acceptable if the image quality was extremely poor and/or if only quasi-circular secondary osteons are selected (i.e., any asymmetric, dumbell, or other circuitous osteons are avoided). But, we have asserted [1] that many of these less typical secondary osteon types should NOT be eliminated because doing so can lead to inaccurate interpretations of load history.

References

1. Skedros et al. (2014) AAPA abstract #107;
2. Crescimanno and Stout (2012) J Forensic Sci 57:287-294;
3. Mears et al. (2014) AAPA abstract #106.

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