A novel specimen-specific methodology to optimise the alignment of long bones for experimental testing

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# Abstract

The choice of coordinate system and alignment of bone will affect the quantification of mechanical properties obtained during in-vitro biomechanical testing. Where these are used in predictive models, such as finite element analysis, the fidelic description of these properties is paramount. Currently in bending and torsional tests, bones are aligned on a pre-defined fixed span based on the reference system marked out. However, large inter-specimen differences have been reported. This suggests a need for the development of a specimen-specific alignment system for use in experimental work. Eleven ovine tibia were used in this study and three-dimensional surface meshes were constructed from micro-Computed Tomography scan images. A novel, semi-automated algorithm was developed and applied to the surface meshes to align the whole bone based on its calculated principal directions. Thereafter, the code isolates the optimised location and length of each bone for experimental testing. This resulted in a lowering of the second moment of area about the chosen bending axis in the central region. More importantly, the optimisation method decreases the irregularity of the shape of the cross-sectional slices as the unbiased estimate of the population coefficient of variation of the second moment of area decreased from a range of (0.210-0.435) to (0.145-0.317) in the longitudinal direction, indicating a minimisation of the product moment, which causes eccentric loading. Thus, this methodology serves as an important pre-step to align the bone for mechanical tests or simulation work, is optimised for each specimen, ensures repeatability, and is general enough to be applied to any long bone.

# Introduction

When characterising material properties of bone at the tissue level, samples are machined into regular specimens to enable the application of Euler-Bernoulli equations to study the effects of applying loads, producing shear stress in torsion and direct stress in bending. Bending and torsion tests are used to characterise the mechanical behaviour of whole bones. In bending, a load is applied laterally while the bone is supported between two external rollers. In torsion, the epiphyses are mounted to enable a torque to be applied along the diaphysis. To study the mechanical behaviour of bone at the structural level, its complex geometry needs to be taken into account.

Currently, anatomical reference frames are used to mount bones for experimental testing in four-point bending and torsion (Cristofolini et al., 1996, 1997, 2000). These experiments have large standard deviations in the results (Cristofolini and Viceconti, 2000). The Ruff and Hayes method (1983) is the most repeatable method known to assign a standard coordinate system for in-vitro testing (Conti et al., 2008) and has been used in structural testing of bones (Finlay et al., 1995; Ebacher et al., 2007; Varghese et al., 2011). The ability of this system to align bones to optimally resist bending and torsion has never been compared to analytical results calculated from solid mechanics. The aims of this study are to:

* develop a method that calculates the principal directions of bone using imaging data,
* compare the second moment of area and its coefficient of variation across the cross-section using the anatomical reference frame and the new method in the principal and anatomical directions, and
* obtain landmarks to define bone alignment for in-vitro testing in four-point bending and torsion.

# Materials and Methods

11 ovine tibiae from 5 months old British Texel lambs weighing approximately 20kg were harvested immediately after slaughter. The bones were cleaned of soft tissues, wrapped in cloth soaked in 1% Phosphate Buffer solution and frozen at -20°C in sealed bags. Bones were thawed and scanned at a resolution of 115 microns in a Metris X-Tek 225 CT System (Nikon Metrology, Tring, UK). Manual segmentation was conducted in Mimics 15.0 (Materialse NV, Leuven, Belgium), to create cortical and endocortical masks. Geomagic Studio 12.0 (3D Systems, North Carolina, USA) was used for repair and smoothing to produce approximately 10,000 triangles for export in STL format.

Euler-Bernoulli objects have a set of principal moments (*I1*, *I2* and *I3*, in descending order), and their corresponding principal axes, where they would experience maximum and minimum stresses in the absence of eccentric loading (Ruff and Hayes, 1983):

|  |  |
| --- | --- |
|  | — (1) |

The volume second moment, *I*, is a measure of the distribution of the material points with respect to the axis. When defined about cartesian axes, they are:

|  |  |
| --- | --- |
|  | — (2) |

When the load to an object is not applied about the principal axis, or if the object is asymmetrical, a product moment of area is experienced:

|  |  |
| --- | --- |
|  | — (3) |

The product and area moments can be assembled to solve for the principal moments *I*:

|  |  |
| --- | --- |
|  | — (4) |

An area moment is maximised when its product moment is minimised. As bone geometry is irregular, the object will be optimised to experience *near* pure shear in torsion, and *near* pure direct stress in bending. Here the principal directions will be referred to as *D1*, *D2* and *D3*, representing the initial coordinate system used in aligning the bone, and corresponding to *I1*, *I2* and *I3*.

The algorithm (Figure 1) to align a bone along its principal axes was developed in RhinoScript and carried out in Rhinoceros (Robert McNeel & Associates, Seattle, USA). Firstly, the two masks were converted to a single solid body, using standard CAD procedures. Then, the volume centroid of the body was calculated to act as the origin for the principal axes. The principal moments and their corresponding axes were computed and sorted in descending order. The bones were then rotated so that their first principal directions coincide with the global axes, using the dot and cross product. This was repeated until the error between the current and target axis equals zero (Figure 1, loop A) and repeated for the second principal axis *D2* (Figure 1, loop B).

In bending, only the span of the bone between the external two rollers and in torsional loading only the free region that is not embedded contribute to the strength of the bone in testing (Ebacher et al., 2007). The location of this free region (and span) for experimental testing was found by minimising the variation in the second moment of area along the segment. Firstly, the first cut, which defines the proximal diaphysis, is allowed to be located between 20-50% of the bone length. The width at this cross-section was used to locate the distal diaphysis at five times the distance of the initial width. (Figure 2A). The widest width throughout the produced segment is then calculated to ensure that the segment fulfils the minimum span-to-width ratio of 4:1, shown by Hardy and Pipelzadeh (1991) to be the minimum required to minimise shear. The segment is then passed through the algorithm again for alignment (Figure 1).

In both the second and third sections of the algorithm, cutting planes are used to section the bones and bone segments at 5% interval along the long axis (Figure 2B). The intersection between the neutral axis and each slice is calculated (black dots). These are used as the reference points for the calculation of the second moment of area.

Code was written to locate the mesh vertices that were furthest away in the first principal axes, to yield the location of six reference landmarks for aligning the bone during experimental testing, with three on each end of the bone (Figure 3). Physically, the landmarks are located with the aid of an optical tracker, to avoid misalignment

Data were not normally distributed and so an unbiased estimate of the population coefficient of variation (*v*) of the second moment of area for each alignment method for each bone was determined. The ratio between the standard deviation and the mean value of the second moment of area of the cross section was calculated, correcting for sample size. Friedman ANOVA was used to test the hypothesis that there is no difference in the *v* of the second moment of area of the bone among the three alignment methods. When the null hypothesis was rejected, the one-tailed paired sample Wilcoxon signed-rank test with Bonferroni correction was used to test the hypothesis that the new alignment method decreased the median *v*, compared to the anatomical alignment.

# Results

The methodology optimises the alignment of long bones by ensuring that *I1*>*I2*>*I3* in the midshaft (Figure 4). By focussing on the diaphyseal section and optimising the bone segment to be used in the analysis, the spread of values decreased (Figure 4).

The three different alignment methods are compared in Figure 5 and Table 1. The second moment of area in the *D1*, *D2* and *D3* directions were different between the three alignment methods (Friedman ANOVA, χ2(2)=20.182, *p*<0.001; χ2(2)=9.455, *p*=0.009; and χ2(2)=16.545, *p*<0.001 respectively; Table 2).

The new methodology resulted in a decrease in *v*. for the anatomical vs. optimised whole group in the *D1* (*p*=0.003) and *D2* (*p*=0.006) directions (Table 2). The decrease was significant in the anatomical vs. optimised segment group (*p*=0.012 and *p*=0.002). Optimising the segment brought significant improvement only in *D2* (*p*=0.0015) direction, and resulted in a curve that is flatter and more symmetrical, with a higher value recorded at the ends when compared to the results obtained using the ‘optimised whole’ algorithm (Figure 5).

The intersection between the principal axis and the cortical bone in each alignment method is shown in Figure 6. Filled dots show the anatomical landmarks that were used (from Finlay et al., 1995). These landmarks are located more anteriorly on the proximal end when the whole bone is aligned based on its geometrical properties and are more medial to the intercondylar eminence when the bone is aligned based on its optimised segment. On the distal end of the tibia, the landmarks shift posteriorly with each alignment method.

# Discussion

A methodology that calculates and uses the initial principal directions of bone as a subject-specific coordinate system, to enable a state of *near* pure bending and torsion during mechanical testing, has been developed. This results in a more even distribution of the second moment of area that is accompanied by a lowering of its coefficient of variation in the *D1* and *D2* directions. Its performance is an improvement on the literature.

The alignment of the whole bone using geometrical properties corresponded well with the results obtained by using an anatomical-by-eye reference system in the middle section (Finlay et al., 1995). The location of the lowest second moment of area was in the diaphysis in both studies (Figure 7).

In all three directions, the second moment of area rises more gradually at the epiphyseal regions in the study here than in Finlay et al. (1995). We found that the second moment of area for our whole ovine tibiae ranged (4,293-37,874mm4) in the *D2* and (3,153-42,461mm4) in the *D3* directions. In contrast, they obtained values of (7,000-70,000mm4) and (4,500-68,000mm4) in the *D2* and *D3* directions, respectively.

In this study the coordinate systems were determined from the volume second moment of the cortex, which is based on its morphology in three-dimensions. However, in all the previous work, the geometrical properties were evaluated on a slice-by-slice basis, a two-dimensional property. Therefore, existing techniques for studying the geometrical properties of bones is more an analysis of how the *Imax* or *Imin* axes vary across the cross-section of the bone, which does not yield further information on how the bone behaves as a structural entity. Indeed Finlay et al. (1995) reported a range of 1.76 to 86.1° of the angle between *Imax* and the anterior-posterior axis in mature Arcott sheep, with the smallest angle at the midshaft and the largest angle at the proximal and distal ends. Therefore, the second moment of area and standard deviations measured in the midshaft are similar between this study and theirs, whereas these values divert greatly at the ends of the bone as the angle between the reference axes used increases.

Other studies used the centroid as the origin for the principle directions (Finlay et al., 1995; Forman et al., 2012; Perz et al., 2014) whereas the neutral axis point was used in this study. The neutral axis refers to the line about which an object experiences no compressive or tensile stress when it is subjected to pure bending, and can be assumed to coincide with the first principal axis. The neutral axis point is obtained by intersecting *D1* with each slice. These two points do not always coincide, and thus *Imax* that is calculated based solely on the slice would deviate from the *I2* that is calculated based on the bone cortex.

Principal moments can be calculated precisely, but perfect alignment is difficult to achieve experimentally. Although a method that uses reverse digitisation has been suggested to increase repeatability, misalignment might still occur, which would result in shifts in the neutral axis. Moreover, the methodology assumes that the samples have fairly homogenous bone density and so care must be taken in interpreting results obtained from samples with inhomogeneous material properties (e.g. osteoporotic) or geometrical properties (e.g. gene knockout in murine long bones may result in different bone geometries). In general, the testing regions, from the calculated optimised segment, should be fairly similar (e.g. in the diaphysis of the bone) for any comparison to be valid in the first instance.

In summary, a new methodology that calculates the alignment of bone based on its principal axes, so that it approximates a state of pure bending and torsion may be achieved in experimental testing, has been developed. This methodology differs from previous work as it accounts for the three-dimensional geometry of the bone and isolates the segment of the bone that is most optimised for experimental testing.

# Conflict of interest statement

The authors declare that no conflict of interest exist in carrying out this study.

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