Mapping Anisotropy of the Proximal Femur for Improved Image-Based Finite Element Analysis

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Mapping Anisotropy of the Proximal Femur for Improved Image-Based Finite Element Analysis

by

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ABSTRACT

Finite element (FE) models of bone derived from clinical quantitative computed tomography (QCT) rely on realistic material properties to accurately predict patient-specific bone strength in vivo. QCT cannot resolve microarchitecture, therefore QCT-based FE models lack the directionality apparent within trabecular bone. Maps of anisotropy were constructed from high-resolution peripheral QCT (HR-pQCT) images of seven femur specimens using a ‘direct mechanics’ method to measure local anisotropy. The resulting directionality reflected all the major structural patterns visible within the microarchitecture of the proximal femur. Principal stiffness directions were interpolated into QCT-based femur models, and whole bone stiffness was calculated for orthotropic and isotropic models in a sideways fall configuration. Comparing model stiffness to experimental data revealed no difference in correlation ($R_{ORTH}^2 = 0.780$, $R_{ISO}^2 = 0.788$). These results suggest that the variability in stiffness explained by anisotropy at the microarchitecture level does not scale to whole bone models for this specific loading configuration.
ACKNOWLEDGMENTS

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TABLE OF CONTENTS

ABSTRACT .................................................. ii
ACKNOWLEDGEMENTS ................................. iii
TABLE OF CONTENTS ................................. iv
LIST OF TABLES ........................................ vi
LIST OF FIGURES ...................................... vii
LIST OF ABBREVIATIONS .............................. xii

CHAPTER ONE: INTRODUCTION ................. 1
  1.1 Motivation ........................................... 1
  1.2 Hypothesis and Specific Aims .............. 3
  1.3 Outline of Thesis ................................. 5

CHAPTER TWO: BACKGROUND AND LITERATURE REVIEW 6
  2.1 Bone Tissue ........................................ 6
    2.1.1 Hierarchy of Bone ............................ 7
    2.1.2 Bone Modeling and Remodeling ........... 9
    2.1.3 Bone Fragility ............................... 12
  2.2 Medical Imaging .................................... 15
    2.2.1 Dual-energy X-Ray Absorptiometry ........ 15
    2.2.2 Computed Tomography ....................... 17
    2.2.3 High-Resolution Peripheral Quantitative Computed Tomography 22
    2.2.4 Quantitative Image Processing ............. 26
  2.3 Biomechanics of Bone ............................ 32
    2.3.1 Bone Strength ................................ 32
    2.3.2 Mechanical Testing .......................... 35
    2.3.3 Finite Element Analysis .................... 38
    2.3.4 Density -Elasticity Relationships ........ 43
    2.3.5 Directionality of Bone Strength .......... 46
  2.4 Summary and Knowledge Gaps .................. 59
CHAPTER THREE: METHODS

3.1 Image Processing
   3.1.1 HR-pQCT Image Processing
   3.1.2 QCT Image Processing
   3.1.3 Image Registration

3.2 Mapping Anisotropy
   3.2.1 Direct Mechanics
   3.2.2 Sensitivity Analysis
   3.2.3 Fabric Tensors
   3.2.4 Map of Anisotropy

3.3 Finite Element Analysis
   3.3.1 Model Construction: Meshing and Material Properties
   3.3.2 Model Behavior: Boundary Conditions and Solutions

CHAPTER FOUR: RESULTS

4.1 Map of Anisotropy
   4.1.1 Trabecular Architecture
   4.1.2 Interpolation
   4.1.3 Directionality of Cortical Bone

4.2 Sensitivity Analysis
   4.2.1 Segmentation Threshold
   4.2.2 Cube Size

4.3 Fabric Tensors
   4.3.1 Trabecular Architecture
   4.3.2 Cortical Architecture
   4.3.3 Sensitivity Analysis

4.4 Finite Element Analysis
   4.4.1 Orthotropic and Isotropic QCT-based FE Models
   4.4.2 Cube Overlap
   4.4.3 Fabric-based FEA
<table>
<thead>
<tr>
<th>SECTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAPTER FIVE: DISCUSSION AND CONCLUSION</td>
<td>113</td>
</tr>
<tr>
<td>5.1 Overview of Findings</td>
<td>113</td>
</tr>
<tr>
<td>5.1.1 Mapping Anisotropy</td>
<td>113</td>
</tr>
<tr>
<td>5.1.2 QCT-based FEA</td>
<td>115</td>
</tr>
<tr>
<td>5.2 Synthesis</td>
<td>118</td>
</tr>
<tr>
<td>5.2.1 Density-Elasticity Relationships</td>
<td>119</td>
</tr>
<tr>
<td>5.2.2 Directionality</td>
<td>121</td>
</tr>
<tr>
<td>5.2.3 Whole Bone Stiffness</td>
<td>123</td>
</tr>
<tr>
<td>5.3 Limitations</td>
<td>125</td>
</tr>
<tr>
<td>5.3.1 Image Processing</td>
<td>125</td>
</tr>
<tr>
<td>5.3.2 Mapping Anisotropy</td>
<td>126</td>
</tr>
<tr>
<td>5.3.3 Finite Element Analysis</td>
<td>127</td>
</tr>
<tr>
<td>5.4 Future Directions</td>
<td>129</td>
</tr>
<tr>
<td>5.4.1 Improved Material Properties</td>
<td>129</td>
</tr>
<tr>
<td>5.4.2 Failure Load Prediction</td>
<td>130</td>
</tr>
<tr>
<td>5.4.3 Potential for Clinical Application</td>
<td>131</td>
</tr>
<tr>
<td>5.5 Conclusion</td>
<td>132</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>135</td>
</tr>
<tr>
<td>APPENDIX A: Additional Maps of Anisotropy</td>
<td>149</td>
</tr>
<tr>
<td>APPENDIX B: Copyright Permission Letters</td>
<td>156</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 2.1: Functional differences between DXA, QCT, and HR-pQCT medical imaging modalities. Adapted from Manske et al., 2010 with permission.

Table 2.2: Architectural parameters used to assess HR-pQCT images with derivations.

Table 2.3: Summary of different density-elasticity relationships derived from experimental data. Adapted from Helgason et al., 2008 with permission.

Table 3.1: Descriptive characteristics of femur specimens

Table 3.2: Summary relationships used to derive orthotropic and isotropic elastic constants as a function of the element apparent density \( \rho \) (g/cm\(^2\)).

Table 4.1: Results of solved QCT-based FEA for orthotropic and isotropic femur models.

Table 4.2: Effect of different levels of HR-pQCT cube overlap (i.e. sampling rate) on QCT-based FE predictions of whole bone stiffness.

Table 4.3: Differences in whole bone stiffness measured using principal directions by direct mechanics and MIL methods.
LIST OF FIGURES

**Figure 2.1:** Schematic of the hierarchy of bone and the organization of tissue architecture. Adapted from Weatherholt et al., 2011 with permission.

**Figure 2.2:** Bone remodeling on the surface of bone tissue, featuring the multicellular unit composed of osteocytes, osteoblasts, and osteoclasts. Adapted from Weatherholt et al., 2011 with permission.

**Figure 2.3:** Simulated bone atrophy of a trabecular specimen from a young donor, 35 years of age at autopsy. The resorption and eventual perforation of trabeculae demonstrate the structural damage caused by osteoporosis. Adapted from Müller, 2003 with permission.

**Figure 2.4:** DXA scans of the hip and spine. Bone geometry is contoured and aBMD is calculated within the region of interest.

**Figure 2.5:** A digital 2D image of patient anatomy is composed of pixels with scalar grey values. A voxel of CT data has the same in-plane resolution as the pixels, with an extra dimension describing slice thickness. Adapted from Bushberg et al., 2002 with permission.

**Figure 2.6:** An x-ray source with fan beam geometry combined with a detector array, rotating around a patient collecting projections of the object. Adapted from Bushberg et al., 2002 with permission.

**Figure 2.7:** Simple (left) and filtered (right) backprojection, showing different projections of the object being smeared along the ray path. The noise is reduced in filtered back projection by convoluting the signal with a specific filter function. Adapted from Bushberg et al., 2002 with permission.

**Figure 2.8:** Clinical CT image of the proximal femur *in vitro*, showing trabecular and cortical compartments, but insufficient resolution to resolve microarchitecture.

**Figure 2.9:** HR-pQCT scan of the same femur specimen as Figure 2.8 at much higher resolution, capable of resolving trabecular microarchitecture *in vitro*.

**Figure 2.10:** Clinical QCT scan with calibration phantom included (left). The density values measured by the scanner are calibrated with a linear function (right) calculated from the known densities of the phantom.

**Figure 2.11:** Example motion artifacts from *in vivo* HR-pQCT scans of the distal radius with gradually increasing image distortion. Adapted from Sode et al., 2011 with permission.

**Figure 2.12:** Segmented HR-pQCT image, using Gaussian filtering and a fixed global threshold.

**Figure 2.13:** Theoretical stress-strain curve and corresponding mechanical terms and regions. Adapted from Einhorn, 1992 with permission.
Figure 2.14: Site-specific mechanical testing of the proximal femur simulating loading during a sideways fall. Adapted from Nishiyama et al., 2013 with permission.

Figure 2.15: Segmented HR-pQCT cube of trabecular bone from the femoral head (left), and corresponding FE model (Right) after direct conversion from voxels to elements. The FE cube then underwent unconfined uniaxial compression and elements are colored by stress magnitude.

Figure 2.16: QCT-based FE model forming a continuum model with heterogeneous material properties based on CT attenuation values.

Figure 2.17: Directionality of bone strength for a trabecular bone cube. The ellipsoidal shape describes the specimen’s orthotropy by forming three planes of symmetry in x, y, and z planes. Adapted from Yang et al., 1999 with permission.

Figure 2.18: Lines of principal stress within the proximal femur, which are aligned with the orientation of the trabecular lattice. Adapted from Einhorn et al., 1992 with permission.

Figure 2.19: Diagram of MIL method, where a linear grid is rotated through the bone fabric and the number of intersections with the bone/marrow interface are tallied (left). For trabecular bone resulting MIL in each direction forms an ellipsoid indicating mechanical orthotropy.

Figure 2.20: Examples of fabric Anisotropy calculations using MIL method (A) and VO method (B). The MIL is determined by rotating a 3D grid within a trabecular volume and counting the number of intersections with the bone/marrow interfaces. The VO is the average orientations of the longest lines of intersection within the bone volume over several 3D points. Adapted from Odgaard, 1997 with permission.

Figure 2.21: Example of direct mechanics method with six uniaxial strain cases, three in compression (top) and three in symmetric shear (bottom).

Figure 2.22: Fourth-order tensor fully defining anisotropic material properties (top), which can be simplified into orthotropic orientation by minimizing diagonal values (bottom).

Figure 2.23: Anisotropy map manually developed from empirically observing architecture orientation from 2mm slices machined from a proximal femur. Adapted from Wirtz et al., 2003 with permission.

Figure 2.24: FE model of the proximal femur with boundary conditions mimicking joint contact forces and seven muscle groups to simulate stress during the gait cycle (left). The resulting directions are derived on a per element basis from the resulting principal stresses (center). Change in von Mises strain values in high stress regions resulting from orthotropic material properties (right). Adapted from San Antonio et al., 2012 with permission.
**Figure 2.2:** Vector field of directional strength for each element of micro CT based FE model of the proximal femur for a healthy (left) and osteoporotic (right) specimen. The direction of strength was derived using the MIL method over a spherical volume of radius 2mm. Adapted from Marangalou et al., 2012 with permission.

**Figure 3.1:** Outline of methodology for mapping anisotropy of HR-pQCT images, and applying anisotropic material properties to QCT-based FE models. The three major components include mapping anisotropy from HR-pQCT images, generating QCT-based FE models of the entire proximal femur, and applying orthotropic material properties to the femur models and validating whole bone stiffness with experimental data.

**Figure 3.2:** Example of 3D rigid image registration between HR-pQCT (red) and QCT (green) images of the proximal femur. First the superior surfaces are aligned (left), then coarse adjustments are manually applied (middle), followed by optimization with an intensity based algorithm (right).

**Figure 3.3:** Segmented HR-pQCT image sub-divided to cubes for local anisotropy measurement. Overlapping cubes not shown. The cube size is approximately to scale.

**Figure 3.4:** Locations of cubes used for sensitivity analysis of threshold and cube size, including regions from the femoral head, neck, and intertrochanteric regions.

**Figure 3.5:** Example grids showing the range of cubes sizes from 3mm (top) to 10 mm (bottom), approximately to scale. The different cube sizes demonstrate this varying amount of trabecular bone used to measure anisotropy. This figure does not visualize the overlapping cube volumes.

**Figure 3.6:** Example of a QCT-based FE model replicating experimental design, including 15 degree internal rotation, 10 degree shaft rotation relative to horizontal, and PMMA caps at femoral head, shaft, and greater trochanter.

**Figure 4.1:** Principal stress lines of trabecular bone (left) compared to HR-pQCT based map of anisotropy (right), in the frontal (top), sagittal (middle), and transverse planes (bottom). Arrows represent the principal direction of strength and are colored by principal stiffness.

**Figure 4.2:** Ward’s triangle represents the intersection of primary and secondary compression lines with the primary tension line (left). Examples of Ward’s triangle within the map of anisotropy for a health femur specimen (right).

**Figure 4.3:** Map of anisotropy represented by ellipsoid glyphs, were each axis of the ellipsoid aligned with the principal directions and scaled by the stiffness in that direction, as calculated by direct mechanics.

**Figure 4.4:** Posterior half of interpolated maps of mechanical anisotropy, where each arrow glyph represents the principal direction interpolated for each QCT element. The internal patterns of architecture are visualized for the specimens with maximum (top), median (middle), and minimum (bottom) T-score to highlight similarities in directionality over a range of aBMD.
Figure 4.5: Osteon orientation adapted from Baca et al., 2007 with permission (left), compared to cortical element orientation determined by the interpolated map of anisotropy (right).

Figure 4.6: Angular deviation between the principal direction of several segmentation thresholds and the “default” direction defined at a threshold of 150 mg HA/cm³, measured at nine locations within the proximal femur.

Figure 4.7: Visualization principal directions as the threshold is increased (left to right) for a single cube from the femoral head (top), neck (middle), and greater trochanter (bottom).

Figure 4.8: Angular deviation between the principal direction of several cube side lengths and the “default” direction defined at a side length of 5 mm, measured at nine locations within the proximal femur.

Figure 4.9: Visualization principal directions as the cube side length is increased (left to right) for a single cube from the femoral head (top), neck (middle), and greater trochanter (bottom).

Figure 4.10: Mid-frontal slices of fabric and mechanical anisotropy maps using MIL method (left), and direct mechanics method (right), for a healthy (top) and osteoporotic specimen (bottom).

Figure 4.11: Directionality of cortical sample, 5 mm in length, from the greater trochanter (A), femoral neck (B,C), head (D), and low-density bone from the femoral neck (E), as determined by direct mechanics (center) and MIL methods (right).

Figure 4.12: Sensitivity analysis of cube threshold (top) and size (bottom) using MIL analysis to determine the principal directions using the eigenvectors of the fabric tensor. The “default” direction defined at a threshold of 150 mg HA/cm³ and cube side length of 5 mm, measured at nine locations within the proximal femur.

Figure 4.13: Comparison of whole bone stiffness between FEA predictions and experimental data, for both orthotropic and isotropic QCT-based FE models.

Figure 4.14: von Mises equivalent stress field for orthotropic (left) and isotropic (right) models for the strongest (top) and weakest (bottom) femur specimens, after 1 mm compressive displacement was applied to the PMMA cap on the greater trochanter.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXA</td>
<td>Dual energy X-ray absorptiometry</td>
</tr>
<tr>
<td>aBMD</td>
<td>areal bone mineral density</td>
</tr>
<tr>
<td>BMD</td>
<td>volumetric bone mineral density</td>
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<tr>
<td>QCT</td>
<td>Quantitative computed tomography</td>
</tr>
<tr>
<td>HR-pQCT</td>
<td>High-resolution peripheral quantitative computed tomography</td>
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<tr>
<td>HA</td>
<td>Hydroxyapatite</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>DA</td>
<td>Degree of anisotropy</td>
</tr>
<tr>
<td>BV/TV</td>
<td>Bone volume / total volume</td>
</tr>
<tr>
<td>Tb.N</td>
<td>Trabecular number</td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>Trabecular separation</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>Trabecular thickness</td>
</tr>
<tr>
<td>Ct.Th</td>
<td>Cortical thickness</td>
</tr>
<tr>
<td>MIL</td>
<td>Mean intercept length</td>
</tr>
<tr>
<td>VO</td>
<td>Volume orientation</td>
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<tr>
<td>RBF</td>
<td>Radial basis function</td>
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</tbody>
</table>
CHAPTER ONE: INTRODUCTION

1.1 Motivation

Osteoporosis is a metabolic bone disease that affects one in two women and one in five men over 50 years old (van Staa et al., 2001). The estimated health-care costs of treating osteoporosis and osteoporosis related fractures was estimated to be $1.9 billion per year in Canada in 2001 (Wirktorowicz et al., 2001) and between $13.7-20.3 billion in the United States in 2005 (Burge et al., 2007). These numbers are also likely to increase with the aging population. In addition to the economic burden, patients suffering from osteoporosis-related fractures suffer intense disability and higher morbidity, often requiring highly invasive surgery to repair the structurally comprised bones.

Osteoporosis is defined by the World Health Organization (WHO) as an aerial bone mineral density (aBMD), measured with dual x-ray absorptiometry (DXA), of more than 2.5 standard deviations below the mean for young, Caucasian women (WHO, 1994). However, it has been shown that around half of incident fractures occur in women with aBMD above the osteoporosis threshold (Stone et al., 2003). This is because aBMD is only a surrogate measure of bone’s mechanical strength, which does not assess the quality of bone architecture. In order to directly calculate patient-specific bone strength the mass, material properties, and architecture must be all be quantified, which cannot be accomplished with a two-dimensional (2D) imaging modality such as DXA.

Quantitative computed tomography (QCT) can be used to determine three-dimensional (3D) bone density and geometry of the entire skeleton in vivo. Studies have shown that architectural parameters derived from QCT, such as cortical volume, trabecular BMD, and cross-
sectional area, vary independently of DXA-based aBMD, but do not significantly improve fracture risk prediction (Bousson et al., 2011; Black et al., 2008). This suggests that volumetric density and cortical geometry do not provide sufficient architectural data for predicting fracture. Other studies also analyzed bone microarchitecture in vivo using high-resolution peripheral quantitative computed tomography (HR-pQCT) at the distal radius and tibia, and found that volumetric BMD, trabecular number, and cortical thickness measurements were significantly different between groups with history of hip fracture and healthy controls (Vico et al., 2008). While HR-pQCT is becoming a promising tool for predicting fracture outcomes, it is limited to peripheral sites and cannot provide a patient-specific prediction of failure load at the hip and spine. Hip fractures are associated with significant increases in patient morbidity and mortality, and are one of the most costly osteoporotic fractures from both an economic and patient perspective (Compston, 2010).

Image-based finite element analysis (FEA) is a numerical method that enables non-destructive mechanical testing of bones, providing direct bone strength calculation from non-invasive medical images. When combined with in vivo images, patient-specific assessment of bone strength can be performed. Several studies have found good agreement between QCT-based FEA and experimental data (Nishiyama et al., 2013; Koivumäki et al., 2012; Dragomir-Daescu et al., 2011), and improved prediction of femoral failure load compared to DXA-based aBMD (Cody et al., 1999). These models form a continuum of elements where stiffness is relative to the element density according to one of many existing mathematical relationships (Helgason et al., 2008). However these models typically assign isotropic material properties, which do not reflect the anisotropy of underlying microarchitecture.

The mechanical strength of the femur is highly direction-dependent, and experiences
more than a two-fold decrease in strength when the loading direction is changed from standing to sideways fall configuration (Keyak, 2001). Sideways hip falls are a common example of a low-impact injury, as 90% of fractures occur during falls (Grisso et al., 1991). Imaged-based FE models rely on accurate material properties to produce realistic mechanical behavior under non-physiological loads, and therefore the model must incorporate the anisotropic material properties to accurately predict the stiffness and failure load of the femur. Several approaches have been developed for applying anisotropic material properties to image-based FEA of the proximal femur (Marangalou et al., 2012; San Antonio et al., 2012; Baca et al., 2008; Peng et al., 2006), but none of these studies have applied architecture-based directionality, for both trabecular and cortical compartments, to QCT-based FE models at clinical resolutions. Furthermore, there is a lack of mechanical validation studies for these advanced anisotropic models, and thus the effect of adding anisotropic material properties on the numerical accuracy of QCT-based FEA remains unknown.

1.2 Hypothesis and Specific Aims

Numerous studies have measured the material properties of bone over the last several decades, still a robust method to calculate the magnitude and orientation of bone anisotropy and apply it to clinical QCT-based FE models has not yet been developed. There is also no consensus in literature whether adding anisotropic material properties to whole bone FE models results in an improvement in stiffness and failure load prediction, which is partly due to a shortage of studies validating anisotropic models with experimental data. In order to address this gap in scientific knowledge, this thesis proposes the following:
Hypothesis

QCT-based FE models of the proximal femur with anisotropic material properties defined according to the bone's microarchitecture improves correlation between FEA predictions of whole bone stiffness and experimental data, when compared to models with isotropic material properties.

Specific Aims

To test this hypothesis, three specific aims (SA) were outlined:

**SA1:** To develop a robust methodology for mapping anisotropy for the proximal femur using the microarchitecture data provided by HR-pQCT. Anisotropy will be measured by dividing the HR-pQCT image into numerous overlapping cubes, and applying the direct mechanics method (Van Rietbergen et al., 1996) to an FE model of each cube.

**SA2:** To interpolate architecture directionality derived from HR-pQCT images of the proximal femur directly into QCT-based FE models. By constructing an interpolation volume from the data points of the map of anisotropy, the principal stiffness directions will be calculated as a function of global position within the femur. To match the global positions of HR-pQCT and QCT images, 3D rigid image registration will align the two femur geometries so that material properties can be directly linked from the high resolution to low resolution images.
SA3: To assign boundary conditions to orthotropic FE models of the proximal femur simulating a sideways hip fall. By mimicking the femur orientation and PMMA caps used in an experimental protocol, the whole bone stiffness of this model can be directly compared to experimental data.

1.3 Outline of Thesis

Chapter Two of this thesis provides a review of the current state of the literature, and discusses the details of bone biology, medical imaging, and biomechanics relevant to addressing the hypothesis and completing the specific aims.

Chapter Three outlines the method for mapping anisotropy of the proximal femur from HR-pQCT image data. This also includes details on image processing techniques, and interpolation methods for assigning directionality to the elements of QCT-based FE models. The material properties and boundary conditions used to solve these models are also described in detail.

Chapter Four presents the results of the maps of anisotropy and sensitivity analyses on the user-selected image processing parameters. Whole bone stiffness predicted by each orthotropic and isotropic QCT-based FE model is presented and compared an experimental gold standard.

Chapter Five summarizes the findings and discusses the results within the context of other studies, while outlining the limitations and future directions of this research. Finally this chapter concludes the thesis with final thoughts on clinical applications of QCT-based FEA.
CHAPTER TWO: BACKGROUND AND LITERATURE REVIEW

2.1 Bone Tissue

Bone’s ability to withstand mechanical stress directly affects nearly every aspect of our daily lives. The skeleton has evolved to serve a primarily mechanical function by providing attachment sites for muscles, acting as levers for motion. The mechanical properties of bone tissue are determined by its material composition and architecture (Currey, 2002). Bone tissue is a natural composite material composed of type I collagen stiffened by crystals of calcium hydroxyapatite (HA). The density of bone tissue is the primary determinant of its mechanical strength, which is related to the proportion of HA within the tissue. This is also described as the degree of mineralization which varies at different skeletal sites, giving rise to the different mechanical properties for specific bones. The structural orientation of the bone tissue also varies across several types of bones, producing distinct loading behavior that typically reflects each bone's specific mechanical function within the body.

The unique architecture within bones provides the remarkable ability to maintain mechanical strength and stiffness, while remaining remarkably lightweight. During loading, bone must remain somewhat flexible in order to store elastic energy through deformation, otherwise the energy will be released through micro-cracking and ultimately by fracture. If bone becomes too flexible, normal loading could result in deformations that exceed the tissue yield strain also resulting in cracking and fracture. The ability of bone to balance the contradicting requirements of stiffness and flexibility is largely dependent on the arrangement of the hierarchal tissue structures.
2.1.1 Hierarchy of Bone

Bone tissue has a complex hierarchical structure (Figure 2.1), with architecture spanning from the submicrostructure on the order of nanometers (the intrinsic lamellae structure), the microstructure on the order of micrometers (Harvesian systems, osteons and trabeculae), and the macrostructure compartments on the order of millimeters (cortical and trabecular bone). This thesis will be primarily concerned with the micro- and macrostructure of bone, and is not focused on the cellular mechanics or chemical composition of bone tissue. Individual bones are composed of two main structural compartments, the dense cortical bone that forms the outer shell, and the porous trabecular bone that forms inner architecture. Cortical bone forms the hard outer shell of all bones, and accounts for 80% of the skeletal bone mass (Bronner and Worrel, 1999). It is found primarily in the diaphysis of long bones, and is composed of numerous overlapping parallel osteons aligned with bone's long axis. The osteons are cylindrical structures composed of concentric lamellae of mineralized collagen, packed in alternating orientation, surrounding the vascular Harvesian canal. The organization of the osteons limits the propagation of micro-cracks through the interstitial bone (Yeni et al., 1997).
Figure 2.1: Schematic of the hierarchy of bone and the organization of tissue architecture. Adapted from Weatherholt et al., 2011 with permission.

The porous trabecular bone has nearly identical chemical compositions as cortical bone, but has a porosity of around 80%, while cortical bone has porosity less than 6% (Fratzl & Weinkamer, 2007; Gibson & Ashby, 1999; Gibson, 2005). The functional units of cancellous bone are the trabeculae, which create an irregular, but highly organized lattice structure commonly found in the epiphyses of long bones and the vertebral column, which commonly experience compressive loads, such as body weight. The trabecular lattice is responsible for mitigating stress concentrations on the articulating surface of the bone by distributing the load from the surface over the dense cortical shell. Individual trabeculae have a size on the order of
75-200μm, and are composed of lamellae arranged longitudinally along the trabeculae (Kregstrup et al., 1983).

2.1.2 Bone Modeling and Remodeling

The primary cells responsible for the growth and resorption of bone tissue are the osteoblasts, osteoclasts, and osteocytes (Figure 2.2). Osteoblasts are derived from mesenchymal stem cells, and work in teams to deposit new lamellar bone. Some of the osteoblasts become embedded in bone matrix that they synthesize, and can further differentiate into osteocytes (Han et al., 2004). The osteocytes are the most numerous cells within bone, and form a dense communication network of cytoplasmic processes throughout the lacunae. This arrangement suggests that osteocytes can detect the material and structural integrity of bone, and could be responsible for signaling surface cells to engage in bone formation or resorption (Parfitt 1996, Verborgt et al., 2000, Bonewald 2007).

Osteoclasts are derived from hematopoietic stem cells and can secrete enzymes that dissolve bone matrix, causing bone resorption. Bone resorption is a necessary process that creates the marrow cavity and forms the cortical and trabecular compartments during bone development. Osteoclasts also remove damaged bone, which must be excavated before the osteoblasts can repair the structure with new bone matrix. Thus during the repair process, the osteoblast and osteoclast form a multicellular unit which must balance the processes of bone formation and resorption in order to preserve chemical and mechanical homeostasis in the body.
Bone modeling occurs during the developmental stage of life, when new bone is deposited without any previous resorption, leading to changes in the size and shape of bone. The purpose of bone modeling is to establish the skeleton's peak bone mass, defined as the amount of bone tissue present at the end of skeletal maturation. Opinions differ on age at which peak bone mass is attained (Krolner and Nielsen, 1982, Recker et al., 1992, Slosman et al., 1994), but it is well understood that progressive bone loss occurs as we age, ultimately leading to late-life fragility. The importance of peak bone mass in late-life bone strength is well documented (Matkovik et al., 1979; Ferrari and Rizzoli, 1998; Dertina et al., 1998; Slemenda, 1990), and it is understood that individuals with relatively high bone mass at a young age will tend to maintain greater bone mass in the later stages of life. Since the majority of bone mass is accumulated
during childhood and adolescence (Rizzoli et al., 2001), it has been suggested that individuals prone to late-life fragility could be identified, even before puberty, by low bone density values for their age (Ferrari and Rizzoli, 1998, Dertina et al., 1998).

Bone remodeling is a life-long process, acting on the trabecular, endocortical, and intracortical surfaces in order to maintain an inherent safety factor that keeps bone strength at an optimal biological level (Biewener, 1993; Alexander 1981). The removal and repair of damage is the most frequent initiator of the bone remodeling process (Taylor, 1997). The continuous cyclic loading of bone gradually fatigues the tissue, which requires the cells to detect the location and degree of damage, remove the damaged bone, deposit new bone matrix, and reconstruct the micro- and macroarchitecture (Parfitt 1996, Martin 2002).

The biological and mechanical factors affecting modeling and remodeling processes are under continuing investigation. One theory regarding the mechanical factors regulating bone suggests that modeling and remodeling are regulated by its loading environment, resulting in an adaptive response in tissue composition and structure (Frost, 1987). In other words, bones undergoing excessive loading will increase in mass through additional bone formation, while unloaded or under loaded bones will lose mass through increased bone resorption. Examples of supporting evidence includes the adaptation in the size and shape of the playing arm of tennis players (Currey 2002), the adaptive mineral:collagen ratio of mice with osteogenesis imperfecta (Bonadio et al., 1993; Kozloff et al., 2004), and the reduction in bone mass in unloaded hindlimbs of growing rats (Morey-Holten and Globus, 1998). Experimental studies have also demonstrated that bone formation in long bones is influenced by strain-rate, frequency, amplitude, duration of loading, and interpolation of rest periods (Ehrlich and Lanyon, 2002).
2.1.3 Bone Fragility

As we age, the rate of bone formation steadily decreases, starting as early as young adulthood when the need for skeletal growth declines (Nishida et al., 1999; Oreffo et al., 1998). Decreased bone formation by osteoblasts, relative to the resorption by osteoclasts, causes a negative balance of the multicellular unit leading to a net bone loss and micro structural damage at each remodeling event (Seeman and Delmas, 2006). While the net bone loss is very small, the problem can be exacerbated by a rapid remodeling rate. Rapid remodeling itself can also be detrimental to bone’s structural integrity, since older denser bone is removed and replaced with younger less mineralized bone, thus reducing its stiffness (Boivin and Meunier, 2002).

Remodeling also creates temporary excavation sites, which become vulnerable stress concentrators on bone surfaces that become predisposed to micro-damage (Currey, 2002).

Remodeling modifies the physical size and shape of whole bones, as well as the interior architecture by adding and removing matrix from the surface of bone. Since the porous trabecular bone presents significantly more surface area than cortical bone, it is considered the more metabolically active compartment, making it sensitive to changes in the remodeling process (Parfitt et al., 1983). The increased remodeling sites per unit volume within trabecular bone makes it vulnerable to rapid remodeling, which can create deep resorption cavities that eventually disrupt trabecular connectivity (Figure 2.3). This loss in connectivity is more detrimental to overall bone strength than trabecular thinning because it hinders the ability of the trabecular lattice to evenly distribute loads from the bone's articulating surfaces to the dense cortical structure (van der Linden et al., 2001).
Figure 2.3: Simulated bone atrophy of a trabecular specimen from a young donor, 35 years of age at autopsy. The resorption and eventual perforation of trabeculae demonstrate the structural damage caused by osteoporosis. Adapted from Müller, 2003 with permission.

As the rate of bone formation decreases with age, continuous bone remodeling steadily reduces the total volume of bone, eventually perforating the trabeculae and gradually thinning the cortical bone, accelerating the rate of structural decay. As pores begin to coalesce within the cortical bone, it becomes more vulnerable to the propagation of cracks, which spread through the interstitial bone. These cracks are mainly resisted at the cement sheath surrounding the secondary osteons, which can deflect certain cracks. Meanwhile, the perforated trabecular lattice loses the ability to share and distribute compressive loads over the cortical shell. The combined reduction in bone mass and architecture impairs bone's mechanical strength, which can lead to severe trauma in the form of cracking or breaking of vital load bearing structures. This degenerative decay is clinically diagnosed first as osteopenia, and as the degeneration increases in severity, it
is finally classified as osteoporosis. Osteoporosis is a silent disease, meaning that it does not present outward symptoms as bone loss continues, gradually diminishing bone strength leading to an increase in fracture incidence across several populations including men (Bilezikian, 1999), premenopausal women (Gourlay and Brown, 2004), and most frequently postmenopausal women (Melton et al., 2009). Osteoporosis is also the most prevalent metabolic bone disease in the western world, affecting one in two women and one in five men over 50 years old (van Staa, 2001).

After several years of degeneration due to osteoporosis, a moderate load sustained from a fall for example, can have catastrophic results and could shatter vulnerable sites in the proximal femur, distal forearm or ankle, or vertebral column. Fracture is the primary clinical consequence of osteoporosis, thus patient-specific fracture risk is the most important factor in treatment selection. Tools for measuring fracture risk, such as FRAX, use several risk factors such as age, sex, BMI, and bone density to provide 10-year risk of osteoporotic fracture. However no universally accepted screening tool exists to identify high-risk patients, and densiometric measurements alone have a low sensitivity to fracture risk. High fracture risk is a consequence of low bone strength, which depends on bone density and architecture. Thus current research is attempting to accurately quantify these multiple parameters in vivo using advanced medical imaging technology. An opportunity also exists to apply engineering methods to directly calculate patient-specific fracture risk using non-invasive numerical techniques based on 3D medical images. A robust and accurate tool for classifying patient-specific fracture risk would maximize the benefits of treatment, by consistently targeting high-risk patients, and could further reduce the incidence of bone fracture in the population (Laupacis et al., 1988).
2.2 Medical Imaging

Advances in medical imaging modalities have unlocked new techniques for non-invasive evaluation of bone mass and architecture. The ability to image bone \textit{in vivo} can sometimes circumvent the need for bone biopsy, where small bone samples are surgically harvested from the body and viewed under a microscope to investigate bone tumors, infections, or other causes of bone pain. This highly invasive procedure risks complications from bruising, bleeding, infection, and bone fracture, and can also leave the patient immobilized for several days. Furthermore biopsy cannot sample the same bone volume twice, which limits prospective studies such as the effects of therapeutic agents. Alternatively, there are several modern imaging modalities available that can measure patient bone density and architecture \textit{in vivo}. While measuring two-dimensional, areal bone mineral density (aBMD; g/cm$^2$) is the current clinical standard for diagnosing osteoporosis, aBMD is only a surrogate measure of bone strength and has a limited ability to predict fracture risk. Scanners that can image the microarchitecture of trabecular bone have shown that architectural parameters are highly predictive of fracture outcomes (Boutroy et al., 2008; Vico et al., 2008).

2.2.1 Dual-energy X-Ray Absorptiometry

The current gold standard for diagnosing osteoporosis is dual-energy x-ray absorptiometry (DXA), which measures aBMD at critical sites such as the proximal femur and lumbar vertebrae (Figure 2.4). The different energy x-rays also enables DXA to distinguish bone and soft tissue. DXA-based aBMD is a widely used surrogate measure of bone strength, because decreasing aBMD is highly correlated with bone fragility. It has been estimated that aBMD predicts approximately 66-74% of the variance of bone strength (Bouxsein et al., 1999).
The World Health Organization defines osteoporosis as a DXA-based aBMD of 2.5 standard deviations below the average Caucasian woman (WHO, 1994). T-scores are typically used to express the number of standard deviations of clinical aBMD measurements relative to a reference aBMD and standard deviation, (i.e. Caucasian women). This differs from a Z-score, which measures the number of standard deviations from a similar population, including similar age, gender, weight, and race. However, aBMD has a low sensitivity to fracture risk, as studies indicate around half of incident fractures occur in women with aBMD above the osteoporosis threshold (Siris et al., 2001; Stone et al., 2003; Schuit et al., 2004). Furthermore, changes in aBMD explain only a small proportion of relative fracture risk reduction of osteoporosis therapy in the vertebrae (Seeman, 2007; Delmas and Seeman, 2004; Delmas, 2000). DXA-based aBMD is unable to distinguish cortical and trabecular compartments or resolve the microarchitecture, which could explain its limited ability to predict bone strength and fracture incidence. Therefore a more sophisticated analysis of bone mass and structure is required to improve in vivo assessment of bone strength and for measuring the effects of therapeutic interventions.

Figure 2.4: DXA scans of the hip and spine. Bone geometry is contoured and aBMD is calculated within the region of interest.
2.2.2 *Computed Tomography*

Computed tomography (CT) is a three-dimensional x-ray based imaging modality that images the inner body in a series of *slices*, or 2D CT images in the transverse plane. The slices are appended together in *stacks*, providing a 3D volume of a region within the body. The basic design involves an x-ray source and detector that rotates around the long axis of the patient, taking multiple images using x-ray transmission at numerous angles. Each measurement of x-ray transmission made by the detector is called a *ray*, and the collection of rays at each angular orientation is called a *projection*. Projections are digital images composed of pixels (picture elements), which become voxels (volume elements) when images are stacked to form a 3D volume. The in-plane dimensions of each voxel are equal to the pixels from the 2D image, while the third dimension corresponds to the slice thickness (Figure 2.5). The pixel values within a projection represent the attenuation of each ray measured by the detector. The degree of x-ray attenuation depends on the linear attenuation coefficient of the material being measured, which is significantly higher in bone, making it easy to identify and extract from the surrounding soft tissue.
**Figure 2.5:** A digital 2D image of patient anatomy is composed of pixels with scalar grey values. A voxel of CT data has the same in-plane resolution as the pixels, with an extra dimension describing slice thickness. Adapted from Bushberg et al., 2002 with permission.

Modern CT x-ray sources use *fan beam geometry*, which emits the rays in diverging angles (Figure 2.6). Each CT image is composed of approximately 800 rays taken at 1000 different projection angles, resulting in 800,000 x-ray measurements (Bushberg et al., 2002). The number of rays and projection angles also determine the spatial resolution of the final image. The x-ray source and detector are then shifted along the z-axis of the scanner and another CT slice is collected. Advances in CT technology, such as multiple detector rings and spiral rotation of the x-ray tube, have significantly decreased the scan times, to less than 10 seconds for an image of the lumbar spine or proximal femur (Engelke et al., 2008; Kalender, 2005).
**Figure 2.6:** An x-ray source with fan beam geometry combined with a detector array, rotating around a patient collecting projections of the object. Adapted from Bushbeg et al., 2002 with permission.

A CT image must be reconstructed from each set of projections before the object can be visualized. A common reconstruction algorithm is *backprojection* which attempts to reverse the acquisition process by smearing each projection onto the image, in the direction parallel to the ray path. The image projections are layered and the CT image is reconstructed, but this method results in significant blurring as the center of the image is oversampled and the edges become noisy due to the smearing. *Filtered backprojection* attempts to correct these errors by convoluting the raw data with a specific filter function in order to reduce blurring (Figure 2.7).
Figure 2.7: Simple (left) and filtered (right) backprojection, showing different projections of the object being smeared along the ray path. The noise is reduced in filtered back projection by convoluting the signal with a specific filter function. Adapted from Bushberg et al., 2002 with permission.

Three-dimensional CT images contain information about bone geometry and architecture, and can evaluate volumetric bone mineral density (BMD; g/cm$^3$) in vivo, throughout the body. The typical in-plane nominal resolution is 200-500 µm, with a slice thickness between 0.5 and 3.0 mm (Figure 2.8). This spatial resolution can distinguish cancellous and cortical bone compartments, but is still unable to resolve individual trabeculae, which have a thickness on the order of 200 µm (Gibson, 2005). The addition of a calibration phantom enables quantitative analysis of bone density, typically measured in mg HA/cm$^3$, and is commonly termed
quantitative CT (QCT). The BMD measured by QCT images correlates well with apparent and ash density of bone (Ciarelli, 1991). However, BMD of trabecular bone only accounts for 40 to 80% of the variance in experimentally determined apparent stiffness modulus of bone (Ciarelli, 1991), and the unexplained variance in strength is likely due to the anisotropic mechanical properties of trabecular bone. Density is only a scalar value that represents the averaged material properties without any directional information. Trabecular specimens with similar density have been shown to exhibit significantly different strengths depending on the organization of the trabecular lattice (Goldstein et al., 1993; Ducheyne et al., 1977). QCT is unable to capture this kind of microarchitectural detail and the volumetric and structural parameters derived from QCT have not significantly improved the prediction of hip fracture compared to DXA-based aBMD measurements (Bousson et al., 2011, Black et al., 2008). These studies suggest that the structural complexity of the proximal femur cannot be captured by QCT, and that additional architectural data are still required to fully characterize whole bone strength.

Figure 2.8: Clinical CT image of the proximal femur in vitro, showing trabecular and cortical compartments, but insufficient resolution to resolve microarchitecture.
2.2.3 High-Resolution Peripheral Quantitative Computed Tomography

A relatively new medical imaging tool was introduced in 2005 called high-resolution peripheral quantitative computed tomography (HR-pQCT), which has enabled in vivo imaging at unprecedented resolutions. HR-pQCT has a nominal isotropic resolution of 82 µm capable of visualizing individual trabeculae (Figure 2.9), providing a more detailed description of bone architecture in vivo compared to clinical QCT. Microarchitecture measurements using HR-pQCT were able to show statistically significant differences between patients with and without fracture, where BMD alone could not (Boutroy et al., 2005). This also provides an example of how detailed analysis of bone architecture in addition to BMD provides a more complete assessment of bone strength, and can improve fracture risk prediction.

HR-pQCT functions on the same principles as clinical QCT, but the enhanced resolution comes at the cost of a smaller field of view. This limits HR-pQCT to in vivo analysis of distal sites on the radius and tibia only, excluding some of the clinically relevant sites at the hip and spine. Research using HR-pQCT has demonstrated successful quantification of bone strength and fracture load at these distal sites (MacNeil and Boyd, 2008a), with acceptable correlations compared to QCT measurements of central sites such as the hip and spine (Liu et al., 2010a; Vico et al., 2008). The smaller, more distal scanning site significantly reduces the radiation exposure from HR-pQCT compared to whole body clinical QCT, with an effective patient dose of approximately 3 μSv per scan (Laib et al., 2004). Additional technical differences between HR-pQCT, clinical QCT, and DXA are summarized in Table 2.1.
Figure 2.9: HR-pQCT scan of the same femur specimen as Figure 2.8 at much higher resolution, capable of resolving trabecular microarchitecture *in vitro.*
Table 2.1: Functional differences between DXA, QCT, and HR-pQCT medical imaging modalities. Adapted from Manske et al., 2010 with permission.

<table>
<thead>
<tr>
<th>Clinical Imaging Site</th>
<th>DXA</th>
<th>Clinical QCT</th>
<th>HR-pQCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Imaging Site</td>
<td>Hip, Spine</td>
<td>Proximal femur, lumbar spine</td>
<td>Distal radius, distal tibia</td>
</tr>
<tr>
<td>Spatial resolution (voxel size)</td>
<td>N/A</td>
<td>~160-500 µm in-plane, non-isotropic</td>
<td>82 µm isotropic</td>
</tr>
<tr>
<td>Outcome Measures</td>
<td>aBMD</td>
<td>BMD, macro-architecture</td>
<td>BMD, microarchitecture</td>
</tr>
<tr>
<td>Reproducibility (%CV) for standard measurements</td>
<td>0.6-1.9%</td>
<td>0.6-4%</td>
<td>1-4.5%</td>
</tr>
<tr>
<td>Typical scan time per site</td>
<td>1-6 min (depends on model, site and scan mode)</td>
<td>~10s</td>
<td>~3 min</td>
</tr>
<tr>
<td>Relative radiation dose</td>
<td>1-6 µSv</td>
<td>~1.5-3 mSv</td>
<td>~5µSv</td>
</tr>
<tr>
<td>Implementation in FE?</td>
<td>No</td>
<td>Continuum level models; load to strength ratio is predictive of fracture</td>
<td>Direct conversion from image voxel to FE elements, predictive of fracture</td>
</tr>
<tr>
<td>Main advantages</td>
<td>Fast, inexpensive, clinically relevant sites</td>
<td>No dedicated instrument required, clinically relevant sites</td>
<td>Low radiation exposure, high resolution</td>
</tr>
<tr>
<td>Main limitations</td>
<td>Measurements influenced by size, 2D only</td>
<td>Ionizing radiation exposure, no microarchitecture</td>
<td>Non-central sites, need for dedicated instrumentation</td>
</tr>
</tbody>
</table>

The primary benefit of HR-pQCT is the microarchitecture information it provides, from which numerous morphometric indices can be derived for a more complete characterization of bone’s structural integrity than BMD alone. However, since the nominal resolution of HR-pQCT images is approximately equal to the individual trabeculae, these morphometric indices are typically derived from measurements that are less sensitive to resolution (Laib et al., 1998). For instance, the volume fraction of trabecular bone (BV/TV) is derived from the BMD within the region of interest (ROI), where a density threshold of 1200 mg HA/cm³ defines fully mineralized bone. The trabecular number (Tb.N) is defined as the average number of trabeculae per unit
length, and is calculated as the inverse of the distance between the mid-axes of the trabecular elements. Other metrics and their derivations, including trabecular thickness, and separation are summarized in Table 2.2. These HR-pQCT measurements are highly correlated with gold standard micro-CT scanners (Liu et al., 2010b; MacNeil and Boyd 2007a), and have a high degree of reproducibility for in vivo measurements of bone density and morphology with precision of <1% and <4.5% respectively (MacNeil and Boyd 2008b).

Table 2.2: Architectural parameters used to assess HR-pQCT images with derivations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abbreviation</th>
<th>Units</th>
<th>HR-pQCT derivation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone volume fraction</td>
<td>BT/TV</td>
<td>N/A</td>
<td>Number of voxels belonging to bone mineral phase compared to total voxels within a volume</td>
<td>Laib et al., 1998</td>
</tr>
<tr>
<td>Trabecular Number</td>
<td>Tb.N</td>
<td>1/mm</td>
<td>The inverse of the mean distance between the trabecular mid-axis</td>
<td>Boutroy et al., 2005</td>
</tr>
<tr>
<td>Trabecular Thickness</td>
<td>Tb.Th</td>
<td>mm</td>
<td>(BV/TV)/Tb.Th</td>
<td>Boutroy et al., 2005</td>
</tr>
<tr>
<td>Trabecular Separation</td>
<td>Tb.Sp</td>
<td>mm</td>
<td>(1-BV/TV)/Tb.N</td>
<td>Boutroy et al., 2005</td>
</tr>
<tr>
<td>Cortical Thickness</td>
<td>Ct.Th</td>
<td>mm</td>
<td>Cortical volume/outer surface area</td>
<td>Boutroy et al., 2005</td>
</tr>
</tbody>
</table>

Several recent studies have examined the efficacy of HR-pQCT measurements at predicting fracture outcomes (Boutroy et al., 2008; Boutroy et al., 2005; Melton et al., 2007; Sornay-Rendu et al., 2007; Vico et al., 2008; Vilayphiou et al., 2010). An important finding was that certain structural parameters can predict fracture incidence independent of aBMD (Sornay-Rendu et al., 2007; Melton et al., 2007). These studies have identified that BMD, Tb.N, and Ct.Th show consistent and significant differences between groups with history of osteoporotic fracture and healthy controls (Melton et al., 2007; Sornay-Rendu et al., 2007; Vico et al., 2008). Furthermore, some groups have found the heterogeneity of trabecular separation (Tb.SpSD) was
able to discriminate fracture groups from controls where aBMD measurements could not
(Boutroy et al., 2005; Sornay-Rendu et al., 2007). Combinations of structural parameters were
also compared using principal component analysis, which indicated that BMD and cortical bone
thickness explain the majority of variance, while most trabecular indices comprised the second
principal component (Boutroy et al., 2008; Vilayphiou et al., 2010). Although these results
appear promising for future clinical applications of HR-pQCT, additional quantitative analysis of
these high resolution images may yield a more direct approach for calculating bone strength and
fracture load.

2.2.4 Quantitative Image Processing

This thesis utilized clinical QCT and HR-pQCT scanning that both utilize the same
fundamental CT principles, but provide very different spatial resolutions and thus require
different image processing techniques. In this section, the term CT is used to describe concepts
that apply to all CT scanners, while QCT and HR-pQCT refer to specifically to the different
scanning equipment. Once a CT image is acquired and reconstructed, image processing is
required to quantitatively analyze the density and architecture of bone. To determine the amount
of bone mineral phase within a particular ROI the image data must be converted from Hounsfield
units (HU) into milligrams Hydroxyapatite per cubic centimeter (mg HA/cm³). HU describes the
linear attenuation values measured by CT, while mg HA/cm³ describes the physical density of
the tissue within the voxels. Both QCT and HR-pQCT scanners utilize calibration phantoms to
ensure the accuracy of bone density measurements, where the calibration phantom is composed
of a variety of materials with a range of known densities (Figure 2.10). For HR-pQCT, daily or
weekly calibrations are sufficient for multiple scans, whereas clinical QCT scanners typically
require a calibration phantom included within every scan. Clinical QCT scanners can vary the x-ray energy used depending on the amount of tissue between the x-ray source and the detector in order to control radiation dose. Thus a calibration phantom is required to quantify the density of each slice throughout the varied energy levels. For QCT scanning of cadaver specimens without soft tissue a fixed energy level is often sufficient, but a calibration phantom should still be included to ensure accurate density comparison between specimens. The measured CT values of the phantoms can be plotted against the known densities to obtain a linear slope and intercept for converting calibrating density into actual units of mg HA/cm$^3$. This calibration method has shown highly correlated findings between different manufacturers, when comparing several sites within the proximal femur (Ito et al., 2010).

![Figure 2.10: Clinical QCT scan with calibration phantom included (left). The density values measured by the scanner are calibrated with a linear function (right) calculated from the known densities of the phantom.](image)
There are a number technical challenges associated x-ray based CT imaging that can complicate image processing, such as beam hardening, partial volume effects, and motion artifacts. Beam hardening occurs when thicker, more attenuating parts of an object (e.g. cortical bone) attenuate the lower energy photons. This increases the average energy of the x-ray, and decreases the ability to measure low density compartments (e.g. trabecular bone), leading to an under estimation of its density. “Pre-hardening” the beam can correct this problem by filtering out the low energy photons with a layer of copper or aluminum before the object. Partial volume effects occur when a voxel contains two different materials within its volume, such as soft tissue and bone, which creates attenuation values proportional to these two materials. This can severely distort the object geometry if the structure is smaller than the spatial resolution of the scanner, for example when evaluating trabecular bone with QCT. Lastly, motion artifacts occur when an object moves during the scanning procedure (Figure 2.11). High resolution scanners are particularly sensitive to this problem, and should be graded either manually or automatically (Pauchard et al., 2012), and rescanned if motion artifacts are deemed too severe.
Figure 2.11: Example motion artifacts from *in vivo* HR-pQCT scans of the distal radius with gradually increasing image distortion. Adapted from Sode et al., 2011 with permission.

Image segmentation involves the extraction of the bone geometry from the reconstructed CT images. There are a variety of image segmentation methods, which will have a direct effect on the resulting bone density and architecture. The most basic segmentation method uses manually drawn contours to outline the ROI, which can be sped up by interpolating contours between several slices of CT data. Semi-automatic algorithms have also been developed to fit contour lines to the gradient boundaries of the image (Kass et al., 1988; Laib et al., 1998). This method still requires significant manual input and tuning, making it slow and susceptible to human error.

HR-pQCT images can also be segmented by applying a single global threshold that can extract the bone mineral phase of the image based on linear attenuation values or calibrated density values, effectively binarizing each voxel into either bone or nonbone. Dual-thresholding techniques can further segment the cortical and trabecular compartment of HR-pQCT images separately (Buie et al., 2005). The threshold value can also be iteratively adapted to preserve the
same bone volume fraction as the original grayscale image (Pistoia et al., 2001). Since the voxel size is still relatively large compared to the individual trabeculae, this may result in some regions losing connectivity with the main structure, which will be discarded by any modeling software. While this effect is largely dependent on the bone volume fraction of the ROI, thresholding is effective at preserving the mechanical properties of the bone architecture (Laib et al., 1999).

To improve the contrast between trabecular bone and the surrounding bone marrow, the image data is typically filtered before thresholding is applied (Figure 2.12). A 3D Gaussian low-pass filter is commonly used to reduce the noise within the ROI, and smooth the edges of the trabeculae (Pistoia et al., 2003; van Rietbergen 1998; Müller et al., 1994). This method works well when the resolution is sufficiently high such that the contrast between bone and marrow is easily visible (e.g. a voxel size less than 100µm). For less contrasted images, a 3D Laplace-Hamming filter has been used to enhance the edges of the trabeculae, showing good correlation of structural indices with micro-CT data (Laib and Rüegsegger, 1999). This filter defines the structure boundaries as the zero crossings of the second derivative of the gray-scale data, which is calculated in the frequency domain using a Fourier-transformed image. This filter has also shown acceptable reproducibility segmenting in vivo HR-pQCT data (Burghardt et al., 2010; Nishiyama et al., 2009; MacNeil and Boyd et al., 2008b), which typically has less contrast and more noise than cadaver specimens.
Segmenting QCT images is quite a different challenge, as the much larger voxel sizes (i.e. greater than 400 µm) leads to greater partial volume effects. This makes distinguishing trabecular and cortical bone compartments difficult, and it becomes impossible to segment any microarchitecture. Contouring is required to identify the endosteal and periosteal surfaces, and active contouring algorithms can also assist in detecting these edges (Chan and Vese, 2001). Cortical thickness can also be extracted using a technique that relies on a mathematical model of bone anatomy and QCT data, then generates multiple thickness estimates throughout the cortex that are accurate for thickness ranging from 0.3 – 4.0 mm in the proximal femur (Treece et al., 2010).

Figure 2.12: Segmented HR-pQCT image, using Gaussian filtering and a fixed global threshold.
2.3 Biomechanics of Bone

Bones experience constant mechanical strain in a variety complex loading configurations. All loads are a combination of tension, compression, and shear stress. Pure tension rarely causes failure in bone and is much more likely to injure a ligament or tendon. Pure compression is less common, but compressive stress forms a large component of the complex loads applied to weight bearing structures such as the hip and spine. Bending is a frequent loading scenario experienced by long bones that combines tension and compression. The ability of bone to withstand complex loads is a function of the tissue material properties and the architecture. When referring to the tissue properties of bone in this context means properties of the microstructural elements (i.e. Harvesian systems, osteons, trabeculae) composed HA and collagen. The apparent properties of bone refer to the structural properties, which is a more macroscopic description that intrinsically includes the tissue properties, and the organization of the bone architecture.

2.3.1 Bone Strength

Bone tissue relies on sufficient bone mass, with adequate material properties, suitably arranged in space in order to provide necessary mechanical strength to bear loads experienced in daily life (Mosely, 2000). Low levels of stress results in a linear stress-strain relationship and their proportionality is termed the modulus of elasticity or Young’s modulus. The modulus is also the slope of the stress-stress strain curve in the linear region, also known as the elastic region (Figure 2.13). When bone experiences an elastic deformation, it returns to its original shape when unloaded. Once stress exceeds the yielding threshold (i.e. yield strength), the bone will experience plastic deformation and will deform permanently. This non-linear region of the curve is termed the plastic region, where additional stress causes continuous damage to the bone tissue
until the point of failure (i.e. ultimate strength) is reached. The energy stored by the bone up to yielding is termed resilience, and is also the area under the elastic region of the stress-strain curve. The energy absorbed from start to failure is termed toughness, which is measured as the area under the entire stress-strain curve. Osteoporotic bone is typically very brittle, meaning there is minimal plastic deformation because the yield strength and ultimate strength are nearly the same. Certain properties also exhibit strain-rate dependence, and it has been shown that the apparent stiffness and ultimate strength of trabecular bone increase with higher strain-rates (Linde et al. 1991; Carter and Hayes, 1977). A large contributor to this behavior is the biphasic nature of bone, which includes the viscous bone marrow in addition to the solid bone phase. The presence of marrow increases the apparent strength of trabecular bone at the highest strain rates due to the restricted viscous flow of the marrow through the pores within the tissue.

Figure 2.13: Theoretical stress-strain curve and corresponding mechanical terms and regions. Adapted from Einhorn, 1992 with permission.
The geometry and architecture of bone is an important determinant of bone strength, and will directly affect the magnitude of stress and strain experienced under loading. At the tissue level, the mechanical strength of bone is direction dependent, and can be described as an anisotropic material. Studies have repeatedly shown how the anisotropic behavior of trabecular bone is dependent of its porosity and structural orientation (Gibson, 1985; Townsend et al., 1975; Williams and Lewis, 1982). Further studies have looked at quantifying the principal stiffness of the microarchitecture to better understand the directionality of trabecular bone (Van Rietbergen et al., 1996; Goulet et al., 1994; Goldstein et al., 1993; Turner et al., 1990).

The structure of cortical and trabecular compartments has been shown to directly affect apparent bone strength measurements (Liu et al., 2010b; MacNeil and Boyd, 2007b; Sornay-Rendu et al., 2007). Cortical bone is primarily found in long bones where rigidity is needed to resist bending. The cross-sectional area at these sites also plays an important role in resisting bending loads (Melton et al., 1988; Spadaro et al., 1994). For example, the neck of the femur has an elliptical cross section with the long diameter in the inferior-superior direction and greater cortical thickness in the inferior section (Zebaze et al., 2005). The porous trabecular structure is most prominent in weight bearing sites such as the vertebral bodies and the proximal femur, where the number, thickness, and orientation of the trabeculae will determine the ability to withstand the large compressive loads at these sites (Ciarelli et al., 2000; Ulrich et al., 1999; Goulet et al., 1994).

The specialized architecture within certain bones has been described as “well-motivated” for withstanding the specific loads they endure. The early observation that the structure of bone responds to the mechanical environment has been generally attributed to Wolff (1892). This concept has since evolved into theories such as the mechanostat theory (Frost, 1980), which
states that bone grows when loads exceed “normal” magnitudes. These early concepts describe a very simplistic model of the determinants of bone’s apparent properties, by assuming that osteogenesis occurs whenever the correct magnitude of stress is applied. In reality, the architecture of bone is determined by a complex combination of both biological and mechanical factors. For example, the bone cell characteristics and endochondral growth act as biological factors, while body mass and muscle contraction patterns act as mechanical factors. It has also been suggested that the adaptive mechanisms of bone are more responsive to ‘error loads’ induced by high impact unusual directions, rather than the ‘normal’ or functional loading environment. A well cited example is the different osteogenic responses observed in high-impact activities such as tennis and gymnastics compared to low-impact activities such as swimming and cycling (Ehrlich and Lanyon, 2002). Most bones possess some natural curvature that reflects the loads at the epiphyses generated by muscles or body weight, particularly in long bones such as the femur. The architecture of long bones suggests that they are well adapted to resist flexion along its curvature, but it is not immediately clear how this structure would respond to aphysiological loads that do not act along the bone’s natural curvature. While further discussion of the determinants of bone architecture is beyond the scope of this thesis, understanding the complex loading environment and resulting structural properties of bone is crucial for developing accurate numerical models for directly calculating bone strength and fracture load.

2.3.2 Mechanical Testing

Mechanical testing provides the most direct assessment of bone's mechanical properties, and a wide variety of testing methodologies and results have been published in scientific literature. Loading configurations can include compression, tension, and torsion along several
different testing axes, while the scale of bone specimens being testing ranges from small cubes (or cylinders) of trabecular or cortical bone, up to entire bone organs. For large bone specimens, site-specific mechanical testing can be utilized to simulate physiological loads in order to estimate the strength of bone in more realistic loading situations (Langton and Njeh, 2003).

Several studies have used mechanical testing to characterize the mechanical properties of trabecular specimens machined from a variety of weight-bearing locations such as the proximal and distal femur, proximal tibia, and vertebrae (Hvid et al., 1989; Martens et al., 1983; Cater and Hayes, 1977; Schoenfeld et al., 1974; Weaver et al., 1966). It was found that the structural orientation and stiffness of trabecular bone are highly dependent on its anatomical location (Ciarelli et al., 1991). Although bone mass is also strongly correlated with mechanical properties, it can only explain a fraction of the variance in bone strength due to its lack of directional information. This property has been repeatedly demonstrated in the trabecular bone sites, such as the proximal tibia where BMD alone has been reported to explain 35-67% of the variance in apparent stiffness (Ciarelli et al., 1991; Hvid et al., 1989). Other studies have shown that the remaining variance in apparent bone stiffness can be explained by the anisotropy of the trabecular microarchitecture (Ulrich et al., 1999; Müller et al., 2001; Turner et al., 1990), which in this context refers to the direction-dependence of trabecular bone stiffness.

Site-specific mechanical testing is an experimental tool that measures the stiffness and fracture load of whole bones under specific loading configurations. These boundary conditions can reflect physiological loading or traumatic loads (also termed “aphysiogical” or “error loads”) in attempt to simulate an injury. For example, the proximal femur can be loaded in a standing/walking configuration by applying compression to the superior surface of the femur head (Keyak, 2000). Alternatively, the proximal femur can experience a sideways hip fall (Figure
by applying compression to the medial surface of the femoral head and lateral surface of the greater trochanter (Nishiyama et al., 2013; Keyak, 2001). The sideways hip fall is commonly studied loading configuration in the femur due to the high incidence of fractures that occur during these types of falls (Grisso et al., 1991; Nevitt et al., 1989). It has been estimated that the ultimate strength of the proximal femur drops from approximately 5000 N to 2000 N when the loading scenario is changed from a standing configuration to a hip fall configuration (Keyak, 2001). Due to the complexity of both the outer geometry and inner architecture within the proximal femur, we expect the mechanical behavior to be very direction dependent, where the diminished strength in non-physiological loading directions reflects the poorly aligned microarchitecture.

Figure 2.14: Site-specific mechanical testing of the proximal femur simulating loading during a sideways fall. Adapted from Nishiyama et al., 2013 with permission.
While mechanical testing provides the most direct method for quantifying bone’s material properties, there are multiple sources of error that can directly affect the resulting stress/strain measurements. This includes machine compliance, specimen geometry, structural end phenomena, friction at endplates, storage conditions, temperature, continuum assumptions, viscoelasticity, and strain rate dependence (Odgaard, 1997). Since mechanical testing is also destructive, it cannot perform repeated measures on individual specimens. Furthermore, in clinical application these methods would require biopsy of bone which cannot be longitudinally measured for the effects of treatment, and would also present significant pain to the patient, likely immobilizing them for a period of time. Therefore current research relies on numerical simulations to provide non-destructive estimations of bone strength, but often experiments are still required to validate these numerical models.

2.3.3 Finite Element Analysis

Finite element analysis (FEA) finds a numerical solution of a field problem, by determining the spatial distribution of one or more variables. A typical problem involves the distribution of stress over a complex geometric body, but FEA can also be applied fluid dynamics, and electromagnetism problems as well. FEA discretizes a mathematical model into a mesh of simple finite elements, each with specific material properties. Specific loads and boundary conditions are then applied to replicate the physical event being modeled. Determining the stress and strain experienced by each element requires solving partial differential equations that can be done directly or iteratively. Iterative solvers, such as the conjugate gradient method, use a preconditioned matrix that starts with an initial guess of the solution, which is iteratively updated until an acceptable convergence tolerance is reached. This method is not suitable for all
problems, such as when the matrix equations are ill-conditions producing non-convergent results. In these cases a direct method such as the sparse solver is employed, which is much more robust but requires significantly more memory. The iterative solver is advantageous for large scale bone models with over $10^6$ elements due to significant memory savings. In addition to being non-destructive, FEA has the advantage of being able to represent virtually any complex geometry, with custom material properties and boundary conditions. This provides a very versatile approach that can potentially model any physical problem in as much detail as permitted by time and computational resources.

The material properties of FE models can also contain varying degrees of complexity. Simplified models utilize linear-elastic, homogeneous, isotropic material properties in order to provide the fastest solving time for large scale models. Models with linear elastic material properties are computationally efficient, but are unable to simulate plastic deformation or failure of bone. In certain special cases the linear-elastic stiffness has been shown to be linearly proportional to yield strength, which has been demonstrated in trabecular cubes from the proximal femur (Brown and Ferguson, 1980), and slices from the distal radius (MacNeil and Boyd, 2008a). The most direct approach to calculating the post-yield behavior of bone uses elasto-plastic modes that include non-linear material properties that simulates tissue yielding. The elasto-plastic modeling of bone uses significantly more computing power, and is beyond the scope of this thesis.

Image-based FEA is a technique that is particularly suitable for studying the mechanical properties of bone by directly converting image voxels into finite elements, thus generating a mesh directly from image data (Müller and Rüegsegger, 1995; Van Rietbergen et al., 1995). High resolution micro-CT images can resolve the microarchitecture of small trabecular bone
specimens (Figure 2.15), which provides the basis for an FE model to calculate the apparent stiffness and tissue strains (Hollister, 1994). Image-based FEA has been used in a variety of studies quantifying the mechanical behavior of trabecular bone. The apparent anisotropy of trabecular bone has been measured by simulating multiple strain cases to fully define an anisotropic stiffness tensor, from which the principal stiffness directions can be extracted (Van Rietbergen et al., 1996). The numerical accuracy of these simulated strains was also validated with mechanical testing (Van Rietbergen, 1997). Image-based FEA has also been used to back calculate the tissue stiffness of trabecular bone by comparing the resulting apparent stiffness of FE models to experimental mechanical tests (Ladd et al., 1998). Ulrich et al., (1999) used image-based FEA on micro-CT images to show that trabecular anisotropy and architectural indices account for 39% more variance in bone strength compared to BMD alone. Niebur et al., (2000) directly predicted the failure load of cancellous bone using non-linear FE on small cancellous bone samples. Pistoia et al., (2001) compared different imaging modalities for FEA, and demonstrated how certain in-vivo scanners provide adequately accurate bone strength predictions. Thus previous studies have thoroughly demonstrated how FEA can be a versatile research tool, for studying both the apparent and tissue level properties of trabecular bone.
Figure 2.15: Segmented HR-pQCT cube of trabecular bone from the femoral head (left), and corresponding FE model (Right) after direct conversion from voxels to elements. The FE cube then underwent unconfined uniaxial compression and elements are colored by stress magnitude.

Using modern computing technology, recent research has expanded image-based FEA from tissue level studies to the analysis of whole bones to directly calculate mechanical properties such as whole bone stiffness and failure load. These large scale models incorporate much more complexity than models of machined trabecular specimens by including both trabecular and cortical compartments, combined with more realistic boundary conditions. Clinical QCT scanners can provide 3D images of central skeletal sites \textit{in vivo}, at reduced resolution which can also be used in whole bone FEA. However, models constructed from these images do not contain any microarchitecture and could be lacking important structural details. Each QCT voxel represents the average CT attenuation of the underlying structure, leading to large partial volume effects. The attenuation values are then scaled to density values based on the calibration phantom, and elements are assigned a tissue modulus based on one of several
experimentally obtained density-elasticity relationships (Helgason et al., 2008). Thus the QCT-based FE models form a continuum (Figure 2.16) that have provided improvements in bone strength estimations compared to aBMD for the vertebrae (Crawford et al., 2003) and femur (Cody et al., 1999). QCT-based FE has also been used to assess the effect of drug treatment on vertebrae mass and structure, and overall strength (Keaveny et al., 2007). Thus QCT-based FEA is becoming a promising clinical tool for patient-specific estimation of bone strength, especially at critical fracture sites such as the proximal femur and lumbar vertebrae.

**Figure 2.16:** QCT-based FE model forming a continuum model with heterogeneous material properties based on CT attenuation values.
2.3.4 Density-Elasticity Relationships

In order to construct a patient-specific FE model from QCT data, the material properties must be estimated using a mathematical relationship, typically a power function, relating bone’s tissue stiffness to the measured density (Keyak et al., 1990; Keaveny et al., 1993). When compared to mechanical testing, the accuracy of bone strength predictions for whole bone models is highly dependent on the chosen elasticity-modulus functions (Schileo et al., 2007). Yet the derivation of a consistent mathematical relationship with mechanical experiments has proven a difficult task, and a variety of studies have looked at different anatomical sites using different testing methodologies (Table 2.3). In a review of 26 different studies, Linde et al., (1992) found the predicted tissue stiffness of low density trabecular bone, defined as QCT voxels with density of approximately 0.3 g/cm³, varied between 70-673MPa. This variance can be partially attributed to the specific experimental methodology (Helgason et al., 2008), and it is also dependent on different anatomical sites (Keaveny et al., 1997; Morgan et al., 2003). Therefore a patient-specific QCT-based FE model cannot rely on density-elasticity functions averaged from multiple studies (Helgason et al., 2008), and an anatomically accurate relationship must be derived.
Table 2.3: Summary of different density-elasticity relationships derived from experimental data.

Adapted from Helgason et al., 2008 with permission.

<table>
<thead>
<tr>
<th>Study</th>
<th>Site</th>
<th>Type of Bone</th>
<th>Densimetric measure</th>
<th>$\rho$-range</th>
<th>$E$ (GPa)</th>
<th>Geometry (mm)</th>
<th>n</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lotz et al., 1990</td>
<td>Human femoral neck</td>
<td>Trabecular</td>
<td>$\rho_{app}$</td>
<td>0.18–0.95</td>
<td>1.31 * $\rho_{app}^{1.46}$</td>
<td>9 x 5</td>
<td>49</td>
<td>0.91</td>
</tr>
<tr>
<td>Linde et al., 1992</td>
<td>Human proximal tibia</td>
<td>Trabecular</td>
<td>$\rho_{app}$</td>
<td>0.273 Average</td>
<td>4.778 * $\rho_{app}^{1.99}$</td>
<td>7.5 x 7.5</td>
<td>31</td>
<td>0.79</td>
</tr>
<tr>
<td>Keller et al., 1994</td>
<td>Human femur</td>
<td>Cortical &amp; Trabecular</td>
<td>$\rho_{ash}$</td>
<td>0.092–1.221</td>
<td>10.5 * $\rho_{ash}^{2.29}$</td>
<td>8 x 8 x 8</td>
<td>297</td>
<td>0.85</td>
</tr>
<tr>
<td>Keyak et al., 1994</td>
<td>Human proximal tibia</td>
<td>Trabecular</td>
<td>$\rho_{ash}$</td>
<td>0.06–0.27</td>
<td>33.9 * $\rho_{ash}^{2.20}$</td>
<td>15 x 15 x 15</td>
<td>36</td>
<td>0.84</td>
</tr>
<tr>
<td>Morgan et al., 2003</td>
<td>Human femoral neck</td>
<td>Trabecular</td>
<td>$\rho_{app}$</td>
<td>0.26–0.75</td>
<td>6.85 * $\rho_{app}^{1.49}$</td>
<td>8 x 16</td>
<td>27</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Assigning accurate stiffness to QCT elements representing trabecular bone is further complicated by its anisotropic behavior. A solution proposed by Cowin and Yang (1997) extends the density-elasticity relationship beyond a single isotropic power function, into several direction-dependent functions. By analyzing material composition and degree of elastic symmetry separately, it was also shown that the eigenvectors of trabecular bone are independent of volume fraction, while the eigenvalues are not (Cowin and Yang, 1997). Using image-based FE on 141 human trabecular bone cubes without any a priori assumption of elastic symmetry, the trabecular bone was shown to be effectively orthotropic (Yang et al., 1999). In other words, trabecular bone stiffness has three orthogonal planes of symmetry, as opposed to fully anisotropic materials that have different stiffness in all directions. A useful way to visualize orthotropy is using an ellipsoid where each axis is scaled by the material strength in that direction (Figure 2.17). The large specimen database was also used to regress mathematical functions for the apparent Young’s moduli, Shear moduli, and Poisson’s ratio in each plane as a function of specimen bone volume fraction (Yang et al., 1999).
Figure 2.17: Directionality of bone strength for a trabecular bone cube. The ellipsoidal shape describes the specimen’s orthotropy by forming three planes of symmetry in x, y, and z planes. Adapted from Yang et al., 1999 with permission.

2.3.5 Directionality of Bone Strength

One method of assessing accuracy of image-based FEA of the proximal femur is by measuring correlation of model stiffness and strength predictions with experimental data. Recent studies have accounted for up to 90% of the variance in whole bone stiffness using isotropic models (Dragomir-Daescu et al., 2011), but the remaining 10% in stiffness variability has yet determined with isotropic models alone. Thus, current research is attempting to incorporate more realistic material properties to QCT-based FEA of the proximal femur in attempt to better predict the experimentally measured mechanical behavior.

Trabecular bone is an anisotropic material, where the apparent strength and stiffness depends on the loading direction. It has been shown that the directionality of this anisotropic material is primarily a function of the organization trabeculae, and not the tissue level properties.
(Van Rietbergen et al., 1997). In other words, the orientation of principal stiffness for a given volume of trabecular bone is determined mainly by trabecular architecture, and not the bone volume fraction. QCT voxels representing trabecular bone can only quantify the average density within their volume, omitting any of the directionality of the microarchitecture within. Ignoring the directionality of all cancellous QCT voxels could affect the behavior of a whole bone model due to the poor representation of the major trabecular stress lines visible within the proximal femur (Figure 2.18). These structures act as important buttress supports for the cortical shell, by sharing and distributing the compressive loads.

The first step in accounting for trabecular anisotropy is by assigning orthotropic material properties to the cancellous elements of QCT-based FE models. While the elastic constants in each orthogonal plane of describe the relative degree of anisotropy (DA), they still do not contain any inherent information about the directionality of the architecture within that element. Calculating the correct directionality for all the cancellous bone elements of QCT-based models presents a difficult technical challenge that will be the primary focus of this thesis. A robust and repeatable method for determining and applying accurate directionality to anisotropic material properties must be established before any improvement in model accuracy can be measured.
Figure 2.18: Lines of principal stress within the proximal femur, which are aligned with the orientation of the trabecular lattice. Adapted from Einhorn et al., 1992 with permission.

The term fabric was introduced as a purely architectural description of trabecular bone, describing the orientation and alignment of the trabeculae without any information about their tissue properties. Mathematically, the magnitude and orientation of elasticity of an object can be described using tensor. A fabric tensor is a quantitative description the local anisotropy of trabecular bone within a 3D volume using a positive second rank tensor. Combining the eigenvectors of the fabric tensor with the apparent density can fully describe the elastic material properties of any particular volume (Cowin 1985; Cowin 1986). A common approach to measuring fabric is the mean intercept length (MIL) method, which quantifies fabric using the trabecular surface (Harrigan and Mann, 1884; Whitehorse, 1974). Briefly, MIL is calculated by
rotating a linear grid over a large number of 3D angles and measuring the number of intersections with the bone/marrow interface, where the MIL is the average distance between intersections for all the lines of the grid (Figure 2.19). Thus the directions with larger MIL reflect better alignment with trabecular fabric and thus represent a relatively stronger direction. Studies using this method combine density and fabric data to compute mechanical stiffness (Zysset and Curnie, 1995) and predict tissue yield strain (Wolfram et al., 2012), which assumes that the fabric tensors are well aligned with the principal directions of mechanical strength. This assumption was validated by Odgaard and colleagues, who found that the eigenvectors of different fabric tensors were closely related to the principal directions of mechanical strength (Odgaard et al., 1997). This makes intuitive sense because the stiffest directions should correspond to the direction where the individual trabeculae are most aligned. However this was only validated for highly homogeneous trabecular bone from cetaceous vertebral specimens with similar trabecular thickness and separation to human cancellous bone. It is not entirely clear if fabric and mechanical directions remain aligned in non-uniform and highly variable trabecular structures, such as within the proximal femur.
Figure 2.19: Diagram of MIL method, where a linear grid is rotated through the bone fabric and the number of intersections with the bone/marrow interface are tallied (left). For trabecular bone resulting MIL in each direction forms an ellipsoid indicating mechanical orthotropy.

Fabric measures can be either surface based such as the MIL method (Harrigan and Mann, 1984), or volume based such as volume orientation (VO) (Odgaard et al., 1990). The VO method is a statistical measure of a sample of local VOs, which are defined as the direction of the longest intercept line at a particular point within the bone volume (Figure 2.20). Previous studies compared fabric and mechanical principal directions and found that MIL was significantly different from the primary direction of strength despite using homogeneous samples. While the average deviation was small (1.4 degrees), significant deviations did occur for certain samples, with some reaching over 18 degrees in magnitude (Odgaard et al., 1997). The VO method fares slightly better in the primary direction, but had similar difficulty in aligning the secondary and tertiary principal directions (Odgaard et al., 1997). However it is possible that certain samples of trabecular bone exhibit transversely isotropic behavior, which would frustrate attempts to calculate unique secondary and tertiary directions.
Figure 2.20: Examples of fabric Anisotropy calculations using MIL method (A) and VO method (B). The MIL is determined by rotating a 3D grid within a trabecular volume and counting the number of intersections with the bone/marrow interfaces. The VO is the average orientations of the longest lines of intersection within the bone volume over several 3D points. Adapted from Odgaard, 1997 with permission.

The fabric tensor is different from the stiffness tensor in that it only describes the geometric orientation of bone architecture and does not describe tissue level properties. Alternatively, the direct mechanics method directly assesses both the magnitude and orientation of mechanical strength of a trabecular specimen by directly calculating the fourth-order stiffness tensor for a cubic bone specimen (Van Rietbergen et al., 1996). This method uses image-based FEA of trabecular cubes and performs 6 uniaxial strain tests, three in compression and three in symmetric shear (Figure 2.21), to calculate an anisotropic stiffness tensor that fully describes apparent 3D stiffness of that specimen. Since trabecular bone is orthotropic, the tensor can be rotated until the diagonal values are minimized, and the axes of the tensor are aligned with the
orthogonal planes of elastic symmetry (Figure 2.22). The resulting rotation matrix that transforms the tensor into best orthotropic orientation, also describes the principal stiffness direction for that specimen. This method relies on high resolution imaging that can resolve microarchitecture, and uses homogenized tissue modulus for the bone mineral phase. This simplification is permitted since the directionality of bone is determined by the apparent structural properties and not the tissue level properties (Van Rietbergen et al., 1995).

Figure 2.21: Example of direct mechanics method with six uniaxial strain cases, three in compression (top) and three in symmetric shear (bottom).
\[
E = \begin{bmatrix}
e_{11} & e_{12} & e_{13} & e_{14} & e_{15} & e_{16} \\
e_{12} & e_{22} & e_{23} & e_{24} & e_{25} & e_{26} \\
e_{13} & e_{23} & e_{33} & e_{34} & e_{35} & e_{36} \\
e_{14} & e_{24} & e_{34} & e_{44} & e_{45} & e_{46} \\
e_{15} & e_{25} & e_{35} & e_{45} & e_{55} & e_{56} \\
e_{16} & e_{26} & e_{36} & e_{46} & e_{56} & e_{66}
\end{bmatrix}
\]

Anisotropic

\[
E^{\text{Orth}} = \begin{bmatrix}
e_{11} & e_{12} & e_{13} & 0 & 0 & 0 \\
e_{12} & e_{22} & e_{23} & 0 & 0 & 0 \\
e_{13} & e_{23} & e_{33} & 0 & 0 & 0 \\
0 & 0 & 0 & e_{44} & 0 & 0 \\
0 & 0 & 0 & 0 & e_{55} & 0 \\
0 & 0 & 0 & 0 & 0 & e_{66}
\end{bmatrix}
\]

Orthotropic

**Figure 2.22:** Fourth-order tensor fully defining anisotropic material properties (top), which can be simplified into orthotropic orientation by minimizing diagonal values (bottom).

The MIL and direct mechanics methods provide robust calculations of the orientation of material properties for small trabecular specimens, but applying this directionality to low resolution QCT-FE models is another challenge altogether. Studies up to now have had difficulties accurately mapping the structural directionality of the proximal femur. Peng et al., (2006) applied orthotropic properties to elements representing trabecular bone, but made no attempt to align the orthotropic directions with the orientation of the trabecular architecture, and instead left each element aligned with the anatomical axis. Other studies have approached the task of applying directionality to orthotropic material properties in different experimental and computational ways. Early attempts involved cutting away 2mm slices of the femur and manually selecting principal directions based on the observed cortical and trabecular patterns (Wirtz et al., 2003). The directions obtained from each slice were back projected onto the QCT-
based FE model and orthotropic orientation was assigned to the centroid of each element (Figure 2.23). Another study looked only at mapping the principal directions of cortical bone by grinding down the superficial lamellae on the surface of the neck and trochanter and staining with India ink to visualize the vascular canals within Harvesian systems, and again manually assigning principal directions based on the observed osteon orientation (Baca et al., 2008). This study also ignored the directionality of trabecular microarchitecture by assigning cancellous elements an isotropic stiffness. These methods were both very labor intensive and involved a high degree of user bias when observing the architecture orientation and manually translating the directionality to elements within the FE models. Furthermore, the consistency and repeatability of their methods was not reported and would likely be difficult to reproduce. Ideally, a method for assigning directionality to FE models would be automated and robust, and thus much more repeatable.
A more recent study developed a map of femur directionality by assuming the principal stiffness was well aligned with the resulting principal stress after a complex “multi-load” scenario was applied (San Antonio et al., 2012). The boundary conditions of each multi-load included joint contact forces and seven different muscle groups that were designed to simulate stress experienced by the femur during the gait cycle. The resulting principal stress directions did reflect the visible directionality of bone architecture, but certain key transversal structures in the head and greater trochanter were absent (Figure 2.24). This load-specific directionality is a direct
result of the “multi-load” scenario, and the missing transverse structures suggest that either the “multi-load” provides an incomplete loading environment, or that the missing transverse structures are not motivated by physiological loads. Nevertheless, this mapping method has the advantage of being a patient-specific approach, since all directionality calculations came directly from clinical QCT data. However a more robust and sophisticated method for simulation of in vivo boundary conditions would need to be developed and validated with mechanical testing before approaching any clinical application.

Figure 2.24: FE model of the proximal femur with boundary conditions mimicking joint contact forces and seven muscle groups to simulate stress during the gait cycle (left). The resulting directions are derived on a per element basis from the resulting principal stresses (center). Change in von Mises strain values in high stress regions resulting from orthotropic material properties (right). Adapted from San Antonio et al., 2012 with permission.
Several studies have used the maximum von Mises stress to compare differences in mechanical behavior caused by different material properties within QCT-based femur models (Peng et al., 2006; Baca et al., 2008; San Antonio et al., 2012). The von Mises stress ($\sigma'$) is defined as follows with respect to principal axes,

$$
\sigma' = \frac{1}{\sqrt{2}} \sqrt{(\sigma_3 - \sigma_2)^2 + (\sigma_2 - \sigma_1)^2 + (\sigma_1 - \sigma_3)^2}
$$

and also with respect to non-principal axes,

$$
\sigma' = \frac{1}{\sqrt{2}} \sqrt{(\sigma_x - \sigma_y)^2 + (\sigma_y - \sigma_z)^2 + (\sigma_z - \sigma_x)^2 + 6(\tau_{xy}^2 + \tau_{yz}^2 + \tau_{zx}^2)^2}
$$

where $\sigma_3$, $\sigma_2$, $\sigma_1$ are the principal stresses, $\sigma_x$, $\sigma_y$, $\sigma_z$ are the normal stresses, and $\tau_{12}$, $\tau_{23}$, $\tau_{31}$ are the shear stresses. A material is said to be yielding when the von Mises stress exceeds the tissue yield stress ($\sigma' \geq \sigma^y$), but this failure criterion does not account for failure caused by hydrostatic stress because it is directly related to the deviatory strain energy. This yield criterion also works best for isotropic, ductile materials such as metals, and may not be appropriate for bone tissue, which is anisotropic and can become brittle in old age. Nevertheless, the von Mises stress is a useful index for comparing stress distributions between specimens since it accounts for complex loads that incorporate a mix of normal and shear stresses. While the increased effective stress caused by combined compressive and tensile forces is also accounted for, the von Mises remains a positive scalar value and cannot be used to visually disguise tension and compressive stress.
It has been reported that orthotropic material properties cause no difference in maximum von Mises stress or strain under physiological loads when compared to isotropic models (Peng et al., 2006; Baca et al., 2008). Yet the maximum von Mises stress does not describe anything about the distribution of stress, and is a poor failure criterion for bone tissue. Furthermore, the material properties of these models still lack realistic directionality reflecting the major trabecular structures visible within the proximal femur. Another study that including directionality within QCT-based femur models observed a similar indifference in maximum von Mises stress or strain, but did measure a 6.2% and 14% difference in local von Mises stress and strain respectively, within the inferior femoral neck (San Antonio et al., 2012). The increased strain within the cortical bone of the inferior neck is noteworthy, since it is a common site of fracture seen both clinically and in experiments.

A recent study by Marangalou and colleagues (2012) measured a fabric tensor of numerous local volumes within a micro-CT FE model (80 µm nominal resolution) using the MIL method over a sphere with a 2mm radius. The volume size measured with MIL had minimal effects on the fabric tensor orientation when the radius was increased from 2 mm to 8 mm (Marangalou et al., 2012). The fabric tensor was combined with the element density to derive a compliance tensor (i.e. the inverse of the stiffness tensor) using a fabric-elasticity relationship proposed by Zysset and Curnier (1995). The fabric tensor of cortical bone was assigned the identity matrix, signifying isotropic material properties. This method was applied to a healthy and osteoporotic femur cadaver, to detect possible variations in architecture caused by bone degeneration (Figure 2.25), since the change in trabecular architecture of the proximal femur has also been previously suggested as a useful indicator for osteoporosis (Singh, 1970). This was a more robust approach to mapping the material properties over large bone models, but was not
applied to clinical QCT-based FE models, and still lacks experimental validation.

Figure 2.25: Vector field of directional strength for each element of micro CT based FE model of the proximal femur for a healthy (left) and osteoporotic (right) specimen. The direction of strength was derived using the MIL method over a spherical volume of radius 2mm. Adapted from Marangalou et al., 2012 with permission.

As more computing power becomes readily available, we see a transition from labor intensive manual methods to sophisticated computer models for determining the directionality of bone architecture. The main advantages of a computational approach are the superior speed and repeatability compared to manual methods. Lastly, none of the studies discussed thus far have made any attempt to compare the results of their orthotropic models to an experimental gold standard. Thus it remains unclear whether adding anisotropic material properties actually improves the numerical accuracy of to QCT-based FEA of the proximal femur.
2.4 Summary and Knowledge Gaps

A robust and accurate method for quantifying bone strength could have important clinical implications for assessing degenerative bone disease such as osteoporosis by providing the ability to identify patients with weak bones prior to fracture. Bone strength is a function of the tissue mass, material properties, and architecture which cannot be assessed by 2D imaging modalities such as DXA. HR-pQCT is capable of resolving the trabecular microarchitecture, but can only be applied to the distal radius and tibia in vivo. QCT has the ability to quantify 3D bone density and geometry of central skeletal sites such as the hip and spine in vivo, but lacks the spatial resolution to resolve trabecular microarchitecture. Thus QCT-based FEA omits certain structural details, which frustrates the process of assigning realistic material properties and may reduce the accuracy of bone strength predictions. As image-based FEA gradually moves into clinical application, we need to ensure that we are using realistic material properties in order to make accurate predictions of patient-specific bone strength.

Several methods exist in literature for determining and applying orthotropic material properties to image-based FE models of the proximal femur. As a result, these models exhibit very different mechanical behavior leading to several different conclusions regarding the necessity for anisotropic material properties for accurate bone strength estimations. The most recent study found that orthotropic material properties improved the apparent stiffness prediction of a continuum FE model for a single osteoporotic femur specimen relative to a micro-CT gold standard (Marangalou et al., 2012). Although this provides some evidence of the possible benefits of anisotropic material properties, a larger sample size is still required to make conclusions regarding any consistent improvement in model accuracy.

The current state of research is unable to definitively answer whether adding anisotropic
material properties to large QCT-based FE models improve the accuracy of bone strength predictions. To answer this question, a robust method for applying accurate orthotropic material properties to several QCT-based FE models is required. Models composed of complex anisotropic material properties also require significantly more computing power. Therefore experimental data would be greatly beneficial for determining whether the added complexity actually amounts to an improvement in numerical accuracy. This thesis addresses this knowledge gap by comparing orthotropic and isotropic QCT-based FE models of the proximal femur to experimental data to detect differences in correlation between models. A novel approach for mapping anisotropy is also presented in this study, and the principal stiffness directions are interpolated from this map into the orthotropic model in an attempt to better understand the role of material directionality in estimating whole bone stiffness.
CHAPTER THREE: METHODS

This chapter describes the methods and materials used for mapping anisotropy of HR-pQCT images of the proximal femur, and applying directionality from this map into QCT-based FE models. This methodology combines several different image processing and numerical modeling tools to construct the map that will be discussed step by step. The majority of the individual components were developed by others, but their combination in this work is novel. The image processing software, image registration software, interpolation software, and finite element solver were all designed and written by third-parties. The numerical methods for calculating both stiffness and fabric tensors were taken from scientific literature. Also, the values for tissue stiffness were taken from previous mechanical validation studies with large sample sizes. Lastly, the experimental data used as the gold standard for predicting whole bone stiffness of the proximal femur was taken from previously published work. The primary original concept presented here is the mapping of anisotropy from HR-pQCT images, and the interpolation of directionality to QCT-based FE models. For reference, an outline of the components discussed in this chapter and the order of interaction is conveniently summarized in Figure 3.1.
Figure 3.1: Outline of methodology for mapping anisotropy of HR-pQCT images, and applying anisotropic material properties to QCT-based FE models. The three major components include mapping anisotropy from HR-pQCT images, generating QCT-based FE models of the entire proximal femur, and applying orthotropic material properties to the femur models and validating whole bone stiffness with experimental data.
3.1 Image Processing

The first step in image-based FEA of bone was the extraction of bone geometry and density from medical imaging data. While there are numerous imaging modalities capable of viewing bone within the body, this thesis focused on x-ray based CT. The high attenuation of bone tissue makes it easy to identify from surrounding soft tissues, giving CT a distinct advantage in bone imaging. There are two CT modalities utilized in this work, clinical QCT and HR-pQCT, which provide very different spatial resolutions. It follows that the image processing techniques for QCT and HR-pQCT will be approached differently.

Seven female femur specimens were fresh frozen with all soft tissue removed, and stored in saline solution prior to scanning by DXA, QCT and HR-pQCT. Descriptive characteristics for each specimen can be found in Table 1. Femoral neck aBMD was measured by DXA (Hologic QDR4500, Hologic; Bedford, MA), but DXA data for one femur specimens was unavailable.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Age</th>
<th>Side</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>aBMD (g/cm²)</th>
<th>T-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>L</td>
<td>59</td>
<td>158</td>
<td>0.828</td>
<td>-0.934</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
<td>R</td>
<td>59</td>
<td>158</td>
<td>0.792</td>
<td>-1.23</td>
</tr>
<tr>
<td>3</td>
<td>83</td>
<td>L</td>
<td>NA</td>
<td>158</td>
<td>0.717</td>
<td>-3.43</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>L</td>
<td>75</td>
<td>165</td>
<td>0.523</td>
<td>-5.83</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>R</td>
<td>75</td>
<td>165</td>
<td>0.230</td>
<td>-4.74</td>
</tr>
<tr>
<td>6</td>
<td>95</td>
<td>R</td>
<td>50</td>
<td>158</td>
<td>0.515</td>
<td>-3.5</td>
</tr>
<tr>
<td>7</td>
<td>95</td>
<td>L</td>
<td>50</td>
<td>158</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

3.1.1 HR-pQCT Image Processing

HR-pQCT scanning used default human in vivo scanning protocol, but with an extended integration time to increase the duration of observation at each frame (XtremeCT, Scanco
Medical, Brüttisellen, Switzerland; 60 kVp, 1000 µA, 250 ms integration time, 750 projections). Images were reconstructed with 82 µm isotropic voxel size capable of resolving individual trabeculae, which have a thickness on the order of 200 µm. The thinnest trabeculae are represented by a width of approximately 2-4 voxels, where the actual boundary of the trabecula is distorted by partial volume effects, which complicates image segmentation. To improve the identification of the bone/marrow interfaces, different filtering techniques are typically applied to the image data prior to segmentation. A moderate low-pass filter is a robust approach for reducing noise and smoothing any jagged trabecular edges in high resolution images (Pistoia et al., 2001; Van Rietbergen et al., 1998). This study applied a Gaussian filter, which uses convolution-based smoothing that blurs the image, removing high frequency spatial components. A standard deviation (sigma) of 1.2 with a support of 1 (Pistoia et al., 2001) provided sufficient smoothing to the gray-scale image to clearly identify trabecular edges.

An alternative approach was considered, that uses a Laplace-Hamming filter which Fourier-transforms the image into the spatial frequency domain and uses zero-crossings to detect trabecular edges, and a Hamming window to reduce high frequency noise (Laib and Rüegsegger, 1999a). The Laplace-Hamming filter is much more computationally expensive, and was to found to be impractical for micro-CT images of whole bones such as the entire proximal femur. The enhanced edge detection this filter provides is ideal for in vivo images of clinical measurement sites at the distal tibia and radius (Laib and Rüegsegger 1998), where the bone is surrounded by fluid and soft tissue that reduces the contrast of the microarchitecture within. For the dry cadaver specimens used in this study, the soft tissue was removed and the contrast is much higher, and thus additional edge enhancement was not required.

After the image data was filtered, the bone mineral phase was extracted by applying a
global threshold that binarizes the image into either bone or marrow. Selecting a threshold that differentiates bone from nonbone tissue relies on prior knowledge of the differences in density. The partial volume effects at the edges of thin trabeculae can interfere with this estimation by reducing density and geometry that would otherwise be visible with superior spatial resolutions. Therefore the threshold value used to extract bone geometry was carefully considered to best preserve bone architecture. This study used a straightforward approach by means of a single fixed threshold that was determined by examining a range of different threshold values and visually inspecting which value best preserved trabecular connectivity compared to the gray-scale image. A threshold of 136.5 mg HA/cm$^3$ was selected, which represents 11.3% of the density of fully mineralized trabecular bone. By attempting to preserve trabecular connectivity, it is likely that this threshold overestimates the bone volume in high density areas such as the femoral head and cortical compartments. Nevertheless, it was essential that maximum connectivity was preserved during segmentation since the trabecular architecture within the HR-pQCT image data provides the basis of anisotropy calculations.

The primary concern with using a fixed threshold was the effect of partial volumes on trabecular bone geometry, which enlarges the thick trabeculae and shrinks the thinner ones. This causes an artificially high variance in trabecular density and can even disconnect small trabeculae when the thickness approaches the size of the voxels. Some studies attempt to justify the perforation of smaller trabeculae on the grounds that they do not offer much mechanical support to begin with, and thus will not affect overall stiffness estimates (Pistoia et al., 2001). This concept was only assumed valid when the bone volume fraction of the segmented image is approximately equal that of the gray-scale image. Another concern with using a single threshold is the homogenization of density variations within a single specimen by fixing all bone voxels to
a single density value. This effectively ignores the structural and mechanical differences between the trabecular and cortical bone compartments by assigning them the same material properties. A solution to this problem has been proposed using a dual-threshold technique that automatically segments cortical and trabecular bone volumes, enabling different material assignment and separate analysis of each compartment (Buie et al., 2007). This method has been effective for processing clinical measurements at the distal radius and tibia, but has not yet been rigorously tested on large human bones with complex geometries such as the proximal femur. A separate threshold level for trabecular and cortical bone could overcome the challenge in balancing trabecular connectivity with accurate bone volume fraction (BV/TV). Lastly, the resulting volume fraction is highly sensitive to the selected threshold, which ultimately affects the stiffness of a bone specimen. While the effect of threshold has not been thoroughly investigated on modeling outcomes, it has been shown that the orientation of local eigenvectors is not sensitive to the bone volume fraction of the specimen being analyzed (Yang et al., 1999).

3.1.2 QCT Image Processing

Clinical QCT provides a spatial resolution on the order of 0.625 mm, which is sufficient for visualizing cancellous and cortical bone compartments, but cannot resolve the microarchitecture. QCT images were reconstructed with a slice thickness of 0.625 mm and an in-plane resolution of 0.439 mm x 0.439 mm (GE Discovery CT750HD, GE Healthcare, Little Chalfont, UK; 120kVp, 60mAs, 512×512 matrix size). This study segmented QCT images by generating semi-automated contours of the periosteal surface (Stradwin 4.3; Cambridge, UK) (Treece et al., 2010), and stenciling out the femur geometry by setting the background data to zero. Image data was calibrated into real density values using a calibration phantom included in
every scan, and in-house software developed with the Visualization Toolkit (VTK 5.10; Kitware Inc.; Clifton Park, NY). The original image voxels were resliced into 0.625 mm isotropic voxels using cubic interpolation (McErlain et al., 2012). This was a robust approach for segmenting complex geometries such as the proximal femur, without losing any inner image data that would occur from thresholding. However, this method does not immediately distinguish cortical and trabecular bone, and attempts to separate bone compartments based on density were again frustrated by partial volume effects, which are even more severe at clinical resolutions. As a result, density-based thresholding techniques are also unreliable for identifying cortical bone thickness at these resolutions (Hangartner, 2007).

3.1.3 Image Registration

Image registration is a tool for calculating a geometric transformation that aligns points of one object to the corresponding points of another object, or another view of the same object. This study used 3D rigid image registration (Insight Toolkit 3.20, Kitware Inc., Clifton Park, NY) to align HR-pQCT and QCT images of the proximal femur in order to map anisotropy data provided by HR-pQCT onto FE models generated from QCT images. Registration of limb images is common for orthopedic interventions, particularly at the femur, including positioning for robotic surgery (Lea et al., 1994). This typically involves comparing intraspecimen images of bone from x-ray based modalities, which almost exclusively relies on rigid transformations since no scaling or deformation is required (Maitz and Viergever, 1998). This method was classified as semi-interactive because some manual translations were required to align the superior surface of the femurs prior to registration. Following manual alignment, an intensity-based registration algorithm was implemented that iteratively optimizes a similarity measure calculated from the
scalar intensities of all the voxels. This method has the advantage of being highly automated, and requires much less user interaction compared to point-based or surface-based registration algorithms. The image data must be pre-processed before using intensity-based registration, and typically some smoothing is required to eliminate noise that would otherwise increase the solving time. Similar to other 3D CT registration techniques, this method works best when the angular misalignment between images is less than five degrees (Münch and Rüegsegger, 1993), and typically the user must manually apply the coarser image alignments prior to registration (Figure 3.2).

**Figure 3.2:** Example of 3D rigid image registration between HR-pQCT (red) and QCT (green) images of the proximal femur. First the superior surfaces are aligned (left), then coarse adjustments are manually applied (middle), followed by optimization with an intensity based algorithm (right).
3.2 Mapping Anisotropy

All anisotropy analyses were performed on segmented HR-pQCT images of the proximal femur by sub-dividing the image into thousands of overlapping cubes, where each cube provides a single local anisotropy measurement. A 2D example of the sub-division of HR-pQCT images is provided in Figure 3.3. This thesis quantified local anisotropy both stiffness and fabric tensors. The former provides a mechanical description of strength, while the latter is a purely architectural measure of structural orientation. It has been demonstrated that fabric and mechanical tensors are generally well aligned, but this has not been tested for the complex trabecular architecture within the proximal femur, where the bone volume and trabecular orientation are highly variable.

![Segmented HR-pQCT image sub-divided to cubes for local anisotropy measurement.
Overlapping cubes not shown. The cube size is approximately to scale.](image)

**Figure 3.3:** Segmented HR-pQCT image sub-divided to cubes for local anisotropy measurement.
3.2.1 Direct Mechanics

The apparent stiffness matrix describing mechanical anisotropy of each HR-pQCT cube was calculated using the so-called ‘direct mechanics’ method proposed by van Rietbergen and colleagues (van Rietbergen et al., 1996). This was achieved by directly converting voxels of segmented image data into eight-node hexahedral elements that were assigned a homogeneous, isotropic tissue modulus (van Rietbergen et al., 1995). While the lamellar tissue organization is not isotropic, the trabeculae are primarily loaded in either compression or bending, and thus the longitudinal modulus determines the effective mechanical strength for these in situ tests. It has been shown that the apparent anisotropic mechanical behavior of trabecular bone is well predicted by models using isotropic tissue properties, suggesting that the anisotropy of trabecular bone is primarily due to its architecture and not the tissue level properties (van Rietbergen et al., 1997).

The orientation of the anisotropic material properties of each cube was determined by rotating the specimen’s apparent stiffness matrix (Eq 1) such that the non-orthotropic terms are minimized by way of an objective function (Eq 2) (Van Rietbergen et al., 1996).

\[
E = \begin{bmatrix}
  e_{11} & e_{12} & e_{13} & \delta_{14} & \delta_{15} & \delta_{16} \\
  e_{12} & e_{22} & e_{23} & \delta_{24} & \delta_{25} & \delta_{26} \\
  e_{13} & e_{23} & e_{33} & \delta_{34} & \delta_{35} & \delta_{36} \\
  \delta_{14} & \delta_{24} & \delta_{34} & e_{44} & \delta_{45} & \delta_{46} \\
  \delta_{15} & \delta_{25} & \delta_{35} & \delta_{45} & e_{55} & \delta_{56} \\
  \delta_{16} & \delta_{26} & \delta_{36} & \delta_{46} & \delta_{56} & e_{66}
\end{bmatrix}
\] (1)

\[
Obj = \frac{\sum_{i,j} \delta_{ij}^2}{\sum_{i,j} e_{ij}^2}, \quad i,j = 1, ..., 6
\] (2)

Where \(E\) is the anisotropic stiffness matrix determined by six uniaxial strain cases, \(e_{ij}\) and \(d_{ij}\) represent the orthotropic and non-orthotropic terms respectively, and Obj is the objective
function being minimized by rotating $E$. This resulted in a coordinate transform that rotates the specimen into optimal orthotropic orientation, where fully orthotropic materials (Eq 3) result in an objective function of zero. By assuming orthotropic material properties, the rotated stiffness matrix can be inverted to obtain the orthotropic compliance matrix, from which nine orthotropic elastic constants were derived (Eq 4).

$$C = \begin{bmatrix}
C_{11} & C_{12} & C_{13} & 0 & 0 & 0 \\
C_{12} & C_{22} & C_{23} & 0 & 0 & 0 \\
C_{13} & C_{23} & C_{33} & 0 & 0 & 0 \\
0 & 0 & 0 & C_{44} & 0 & 0 \\
0 & 0 & 0 & 0 & C_{55} & 0 \\
0 & 0 & 0 & 0 & 0 & C_{66}
\end{bmatrix}$$ (3)

$$C^{-1} = S = \begin{bmatrix}
S_{11} & S_{12} & S_{13} & 0 & 0 & 0 \\
S_{12} & S_{22} & S_{23} & 0 & 0 & 0 \\
S_{13} & S_{23} & S_{33} & 0 & 0 & 0 \\
0 & 0 & 0 & S_{44} & 0 & 0 \\
0 & 0 & 0 & 0 & S_{55} & 0 \\
0 & 0 & 0 & 0 & 0 & S_{66}
\end{bmatrix} = \begin{bmatrix}
1 & -\nu_{23} & -\nu_{13} & 0 & 0 & 0 \\
-\nu_{32} & 1 & -\nu_{12} & 0 & 0 & 0 \\
-\nu_{31} & -\nu_{21} & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & \frac{1}{G_{21}} & 0 & 0 \\
0 & 0 & 0 & 0 & \frac{1}{G_{13}} & 0 \\
0 & 0 & 0 & 0 & 0 & \frac{1}{G_{32}}
\end{bmatrix}$$ (4)

Where $C$ represents the orthotropic stiffness matrix, $S$ is the corresponding compliance matrix, and $E$, $\nu$, and $G$ are the Young’s modulus, Poisson’s ratio, and Shear modulus respectively. This analysis provides two important results: first, the nine elastic constants describe the degree of anisotropy by fully characterizing mechanical behavior in three orthogonal planes of symmetry. Second, the optimized rotation matrix describes the coordinate transform that rotates each axis from the specimen coordinate system into the planes of orthotropic symmetry, where each row
corresponds to the primary, secondary, and tertiary strength directions. An example of this rotation is provided,

\[
\mathbf{C}^S = \begin{bmatrix}
787 & 154 & 138 & 21 & 147 & -21 \\
154 & 439 & 103 & 47 & 23 & -63 \\
21 & 47 & 44 & 145 & 13 & 31 \\
147 & 23 & 67 & 12 & 248 & 19 \\
-21 & -5.3 & 15 & 31 & 19 & 236
\end{bmatrix}
\rightarrow
\mathbf{R} = \begin{bmatrix}
0.913 & 0.037 & 0.407 \\
-0.200 & 0.910 & 0.365 \\
-0.357 & -0.415 & 0.837
\end{bmatrix}
\rightarrow
\mathbf{C}^O = \begin{bmatrix}
934 & 172 & 76 & -9.0 & 6.5 & 5.2 \\
172 & 481 & 93 & 0.64 & -0.78 & -5.5 \\
76 & 93 & 305 & 15 & -17 & 12 \\
-9.0 & 0.638 & 15.3 & 137 & 30 & -7.8 \\
6.5 & -0.78 & -17 & 3.0 & 181 & 1.0 \\
5.2 & -5.5 & 12 & -7.8 & 1.0 & 258
\end{bmatrix}
\]

\[E_3 = 637 \text{ MPa, } E_2 = 383 \text{ MPa, } E_1 = 332 \text{ MPa, } G_{12} = 133 \text{ MPa, } G_{13} = 214 \text{ MPa, } G_{32} = 227 \text{ MPa,}\]

where \(\mathbf{C}^S\) is the apparent stiffness matrix in the specimen coordinate system, \(\mathbf{R}\) is the rotation matrix where each row corresponds to each plane of orthotropic symmetry, and \(\mathbf{C}^O\) is the apparent stiffness of the same specimen rotated into optimized orthotropic orientation. The resulting orthotropic elastic constants are derived from \(\mathbf{C}^O\), and are sorted in order of magnitude where \(E_3 > E_2 > E_1\).

It has been suggested that image-based modeling is a superior approach for determining anisotropic material properties compared to traditional mechanical experiments (Oggaard, 1997; Van Rietbergen et al., 1996; Yang, 1999). Trabecular bone cubes (or cylinders) machined from cadaver specimens are rarely greater than 5 mm in length, as larger sizes would also sacrifice the number of testable cubes available. Furthermore, compressive testing of small cubes is beset by multiple sources of error such as friction at the end plates, specimen inhomogeneity, and variability due to unaccounted architecture orientation (Keaveny et al., 1997). Other studies report that estimates of stiffness and failure load from compressive tests can have errors up to 40% (Oggaard and Linde, 1991). Direct mechanics analysis using image-based FEA circumvents many experimental limitations by applying consistent and highly controlled boundary conditions.
Furthermore, multiple loading conditions can be performed on a single specimen non-destructively, enabling complete characterization of the apparent anisotropy.

This numerical method for calculating trabecular anisotropy was fully automated, which eliminates all user bias in the anisotropy calculations. There were only a limited number of image processing parameters the affected direct mechanics measurements, including BV/TV resulting from image segmentation, and specimen geometry. As previously mentioned, the BV/TV of a cube specimen has little effect on the principal directions, but does have a scaling effect on resulting model stiffness. The size of the cube analyzed by direct mechanics determines the amount of architecture being analyzed. For the purpose of mapping anisotropy in this study, a small cube size was desirable to effectively increase the resolution of the local anisotropy measurements, while a large cube size would have averaged certain architectural details. The minimum cube size is constrained by the continuum material property assumptions, which becomes suspect at less than five trabecular lengths between interfaces (Harrigan et al., 1988). To satisfy this constraint, this study selected a cube length of 5 mm to capture a sufficient number of trabeculae throughout the proximal femur, except for regions composed of predominantly marrow, which were excluded from direct mechanics analysis.

3.2.2 Sensitivity Analysis

This study performed a sensitivity analysis of the selected HR-pQCT threshold and cube size on direct mechanics results, since these two parameters introduce the most user bias to the mapping process. The threshold value was analyzed first by segmenting a single femur at several levels between the boundaries of obvious over and under thresholding. Direct mechanics was
applied to a small sample of test cubes selected from the femoral head, neck, and intertrochantic region (Figure 3.4) in attempt to assess the effect of threshold at different anatomical locations.

**Figure 3.4:** Locations of cubes used for sensitivity analysis of threshold and cube size, including regions from the femoral head, neck, and intertrochantic regions.

The next sensitivity analysis looked at the effect of different cube sizes on direct mechanics measurements, using the same subset of cubes as the threshold analysis. The cube side length was varied from 10 mm which is larger than most cubes tested in literature, to 3 mm which is just below the minimum size required to satisfy continuum material properties (Harrigan et al., 1988). A 2D depiction of the different cube sizes is provided in Figure 3.5.
Figure 3.5: Example grids showing the range of cubes sizes from 3mm (top) to 10 mm (bottom), approximately to scale. The different cube sizes demonstrate this varying amount of trabecular bone used to measure anisotropy. This figure does not visualize the overlapping cube volumes, which occurred in practice.
A third sensitivity test explored the effect of the overlap between bone cubes (i.e. sampling rate) which directly affects the number of local anisotropy measurements and ultimately the fidelity of the mapping process. A default overlap of 2.15 mm (50% of cube volume) was applied in all three dimensions in order to obtain a large number of cubes and therefore the most detailed anisotropy map possible. Four different maps of anisotropy were generated from an HR-pQCT image of a single femur specimen with overlap of adjacent cubes ranging from 0 mm - 2.5 mm. Four different QCT-based FE models were then constructed using directionality interpolated from the four different maps of anisotropy, and the resulting whole bone stiffness was compared between models.

3.2.3 Fabric Tensors

An alternative approach for quantifying trabecular anisotropy uses a fabric tensor, defined as a positive second rank tensor that describes the 3D alignment bone fabric. In other words, this is a purely architectural measure that quantifies the geometric orientation of the trabecular lattice, but does not quantify the tissue material properties (Cowin 1986). To compare alignment of fabric anisotropy with principal stiffness directions in complex trabecular environments, this study applied the MIL method for all the cubes within the healthiest and most osteoporotic specimens, with T-scores of -1.23 and -5.84 respectively. The resulting fabric-based map of anisotropy was qualitatively compared to mechanical maps of anisotropy. Additionally for these two specimens, orthotropic QCT-based FE models were constructed with directionality determined by both direct mechanics and MIL analysis, in order to compare the effect on resulting whole bone stiffness. Lastly the sensitivity analyses for image threshold and cube size were repeated for MIL measurements.
3.2.4 Map of Anisotropy

The map of anisotropy presented in this work is composed of numerous local measurements of anisotropy, which calculate the orientation of the orthotropic planes of symmetry that represent the principal stiffness directions. Each local anisotropy measurement of a HR-qQCT cube provides a single data point within the map. A qualitative analysis of this map was used to determine whether the patterns of anisotropy agree with the lines of principal stress visible within the trabecular lattice. For the loading conditions applied in this thesis, it was particularly important to observe the transverse structures captured by this map, namely the primary tension line and trochanter stress lines, which will directly affect the apparent stiffness of the whole bone in a sideways loading configuration.

3.3 Finite Element Analysis

FEA of whole bone models of the proximal femur includes three fundamental components: the mesh of elements representing bony geometry, the material properties assigned to the elements, and the boundary conditions applied to analyze mechanical behavior. The models discussed here are based on the clinical QCT images, which were segmented by contouring and not thresholding, resulting in a continuum model. The material properties used in this study were combinations of density-elasticity relationships based on QCT data, and architecture directionality measured by HR-pQCT. An isotropic material model was also constructed to determine the changes induced by anisotropic material properties. Lastly, the models were loaded in a sideways fall configuration and the resulting whole bone stiffness was compared to experimental data collected from a previous study (Nishiyama et al., 2013).
3.3.1 Model Construction: Meshing and Material Properties

The voxels of the QCT image were directly converted into eight-node hexahedral elements, each with a scalar density value. The density provided by the QCT scanner was calibrated using a linear relationship derived from the known densities of the calibration phantom included within each scan. The resulting mesh forms a continuum of elements, which has been shown to provide comparable accuracy to segmented micro-CT models (Verhulp et al., 2006). The element size can directly affect the stress calculations of continuum models, particularly in complicated geometries such as the femoral neck (Yang et al., 2010). Hexahedral elements can result in jagged bone surfaces, but are also less susceptible to instabilities caused by large element size (Ramos and Simoes, 2006). This study used an isotropic element size of 0.625 mm, resulting in models on the order of $10^5$ elements, which provides a relatively smooth outer surface relative the edges of the hexahedral elements.

QCT-based FE models account for the density distribution of trabecular bone by assigning a specific element stiffness using one of many mathematical relationships (Helgason et al., 2008). It is customary that a relationship for Young’s modulus is regressed against the element density in a power function, $E = scalar \times density^{power}$. However, there is no universally accepted approach for selecting the correct density-elasticity relationship, and it is apparent that these functions are region dependent (Morgan et al., 2003), and can result in very different model behavior (Austman et al., 2008). Furthermore these traditional density-elasticity relationships do not account for trabecular anisotropy, which is not captured within QCT elements, resulting in an isotropic tissue modulus being implemented. These assumptions may be valid when physiological loads are considered since the isotropic modulus corresponds to the longitudinal stiffness of the trabeculae, which are well aligned for such loads. When analyzing
non-physiological loads upon the femur, such as a sideways fall, the transverse modulus now becomes a factor, and an isotropic tissue modulus is likely a gross overestimation of the stiffness in this case (Keyak et al., 2001).

To account for the anisotropy within each element of a QCT-based FE model a common approach derives nine orthotropic constants (3 Young’s moduli, 3 shear moduli, 3 Poisson’s ratio) using a mathematical relationship function with the element density (Taylor et al., 2002; Wirtz et al., 2003; Peng et al., 2006, Baca et al., 2008; Yang et al., 2010; San Antonio et al., 2012). This study used the relationships of orthotropic elastic constants determined by Yang and colleagues (1999) as the basis for material table construction. These tissue properties were chosen because they were derived using image-based FEA techniques similar to this study, and applied to micro-CT images of 141 human trabecular bone specimens. Using an in situ analysis similar to direct mechanics, Yang and colleagues demonstrated that trabecular bone is effectively orthotropic without any a priori assumptions of material symmetry (Yang et al., 1999). Following this conclusion, the six eigenvalues for 141 specimens were regressed against their volume fraction using a linear log-log relationship. A compliance matrix was constructed with spectral analysis of the regressed eigenvalues and specimen eigenvectors (Eq 5)

$$\hat{c} = \sum_{k=1}^{6} \Lambda_k N^{(k)} \otimes N^{(k)}, \quad \hat{s} = \sum_{k=1}^{6} \frac{1}{\Lambda_k} N^{(k)} \otimes N^{(k)},$$

where $\hat{c}$ and $\hat{s}$ are the stiffness and compliance tensors respectively, $\Lambda$ is the regressed eigenvalue expressed as a function of specimen BV/TV, and N is the corresponding eigenvector of the specimen. The elastic constants derived from the regressed eigenvalues showed good correlation
with analytical values for all 141 specimens with squared correlation coefficients ($R^2$) between 0.666-0.917 (Yang et al., 1999).

Similar to another study by San Antonio and colleagues (2012), this thesis modified the stiffness in the primary direction of strength with a femur-specific, density-elasticity relationship that was determined from mechanical experiments of 297 femoral trabecular cubes (Kellar, 1994). This tissue stiffness is a function of the dry density of bone, which is has been shown to be more accurate than wet density relationships (Verhulp et al., 2006), and has provided accurate estimation of whole bone stiffness in the proximal femur (Nishiyama et al., 2013). Another study proposed different experimentally derived relationships for trabecular bone at the femoral neck and trochanter, where the bone in the femoral neck was found to be much softer (Morgan et al., 2003). This was only demonstrated with a small sample size of 10-20 cubes per site, and only a limited ability to account for variability due to trabecular architecture alignment during experimentation.

Similar to other studies, an attempt was made to assign different material properties to elements representing purely cortical bone, by identifying these elements with a scalar density greater than 1.2 g/cm$^3$ (Yang et al., 2010; Peng et al., 2006; Keyak et al., 1998). Elements of purely cortical bone were mainly identified in the femoral shaft, where it is thickest. These elements were assigned transversely isotropic elastic constants by making the secondary and tertiary directions identical in magnitude (Dong and Guo, 2004). The relationships used to derive all nine elastic constants for all models are summarized in Table 3.2.
Table 3.2: Summary relationships used to derive orthotropic and isotropic elastic constants as a function of the element apparent density $\rho \, (\text{g/cm}^2)$.

<table>
<thead>
<tr>
<th>Elastic Constant</th>
<th>Orthotropic Models</th>
<th>Isotropic Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cancellous Bone</td>
<td>Cortical Bone</td>
</tr>
<tr>
<td>$E_3$</td>
<td>$10,500,\rho^{2.29}$</td>
<td>$10,500,\rho^{2.29}$</td>
</tr>
<tr>
<td>$E_2$</td>
<td>$E_3 \cdot 0.76,\rho^{0.12}$</td>
<td>$0.57 \cdot E_3$</td>
</tr>
<tr>
<td>$E_1$</td>
<td>$E_3 \cdot 0.47,\rho^{0.09}$</td>
<td>$0.57 \cdot E_3$</td>
</tr>
<tr>
<td>$G_{12}$</td>
<td>$E_3 \cdot 0.26,\rho^{0.24}$</td>
<td>$0.29 \cdot E_3$</td>
</tr>
<tr>
<td>$G_{31}$</td>
<td>$E_3 \cdot 0.45,\rho^{0.18}$</td>
<td>$0.2 \cdot E_3$</td>
</tr>
<tr>
<td>$\nu_{12}$</td>
<td>$0.27,\rho^{-0.09}$</td>
<td>$0.37$</td>
</tr>
<tr>
<td>$\nu_{23}$</td>
<td>$0.14,\rho^{-0.16}$</td>
<td>$0.3$</td>
</tr>
<tr>
<td>$\nu_{31}$</td>
<td>$0.14,\rho^{-0.17}$</td>
<td>$0.3$</td>
</tr>
</tbody>
</table>

Thus far we have a mesh describing femur geometry and density distribution from QCT images, combined with density-elasticity relationships describing the scalar stiffness of each element. The final step was to rotate the stiffness tensor of each element to align with the principal directions of the underlying microarchitecture as determined by direct mechanics analysis of HR-pQCT data. Similar to Marangalou and colleagues (2012), this study mapped the directionality of trabecular bone directly from high resolution images of the microarchitecture. However, instead of applying this map to down scaled images simulating clinical resolution, this study interpolated directionality directly to FE models generated from actual clinical QCT images of the same specimens. This study used radial basis functions (RBF) to interpolate the data points of the map of anisotropy, using a compact (Gaussian) basis function. The uniform spacing of the data permits a fixed interpolating radius which was selected as the minimum distance between points in the interest of computing time. The vectors describing the primary, secondary, and tertiary stiffness directions were interpolated in 3D as a function of global position. In other words, an interpolated volume was constructed that describes the changes
architecture orientation throughout the femur geometry. The orthotropic material properties of each element from the QCT-based FE model were aligned with an interpolated principal direction based on the element’s global position, which was matched to HR-pQCT global position from the previously described image registration.

3.3.2 Model Behavior: Boundary Conditions and Solutions

Orthotropic and isotropic FE models were solved using FAIM (v6.0, Numerics88 Solutions; Calgary Canada) on a desktop workstation using GPU acceleration (Ubuntu v12.04, Canonical Group Ltd, London, UK; 2.66 GHz Intel Xeon X5650, 24GB RAM, 3x nVidia Tesla 6GB). Whole bone stiffness was calculated from the linear models by dividing the resulting reaction forces by the applied displacement. In order to determine whether adding orthotropic material properties improves FEA prediction of whole bone stiffness, a gold standard is required. Typically FEA validation requires an analytical or experimental solution for comparison, but since an analytical solution does not exist, we must rely on experimental data. Mechanical testing of whole femurs is an expensive and time-consuming procedure, but when done properly, the experimental the data is an invaluable reference for our computer models. Whole bone testing also has advantages over cube testing by removing the need for specimen machining, thus preserving all the trabecular and cortical bone architecture and enabling more realistic boundary conditions. Other studies have used FEA of segmented micro-CT models of the proximal femur as a gold standard for analysis of lower resolution continuum models, since micro-CT contains all the microarchitecture responsible for anisotropic behavior (Marangalou et al., 2012, Verhulp et al., 2006). However, comparing one numerical model to another is only valuable if one of them has been fully validated, therefore the majority of studies still rely on experimental
validation for whole bone models (Dragomir-Descaue et al., 2011; Schileo et al., 2007; Keyak et al., 2005).

The resulting whole bone stiffness estimated by the orthotropic and isotropic FE models tested in this study were compared to experimental data collected in a previous study (Nishiyama et al., 2013). The femurs were embedded in PMMA bone cement and loaded in a sideways fall configuration with an internal rotation angle of 15 degrees and the shaft at a 10 degree angle from the horizontal (Courtney et al., 1995). The proximal femur is significantly weaker in a traumatic loading scenario compared to physiological loading configurations such as standing or walking (Keyak et al., 2001). This is because these non-physiological loads test the model’s transverse structures which are typically much weaker than the longitudinal structures, making this the ideal test for studying anisotropic material properties of bone.

The FE models in this study attempted to directly replicate the boundary conditions of the mechanical experiment, by including all rotations and PMMA caps on the femoral shaft, head, and neck (Figure 3.6). The femoral head was fixed in the loading direction with unconstrained transverse motion. The femoral shaft was fully fixed, and a 1.0 mm compressive load was applied to the surface nodes of the trochanter PMMA caps (Figure 3.3). Elements representing the PMMA caps were assigned an isotropic modulus of 2500 MPa and a Poisson’s ratio of 0.3 (Lewis, 1997). During experimentation, the PMMA caps provide the essential fixation to the apparatus, while distributing the loads on the uneven bony surface and preventing any specimen slippage. During modeling, PMMA caps of similar thickness were added to the same locations in order to account for the strain energy they absorbed, and also ensured identical boundary conditions across specimens.
Figure 3.6: Example of a QCT-based FE model replicating experimental design, including 15 degree internal rotation, 10 degree shaft rotation relative to horizontal, and PMMA caps at femoral head, shaft, and greater trochanter.
CHAPTER FOUR: RESULTS

This chapter presents the results in a parallel structure to the methodology described in the previous chapter. First the map of anisotropy calculated from HR-pQCT data is presented and qualitatively compared to the actual architecture visible within the proximal femur. The resulting principal directions interpolated to the QCT-based FE models are also visualized. Next the results of the sensitivity analysis demonstrate the effect of various image thresholds and cube sizes on direct mechanics measurements. Then the results of MIL analysis of anisotropy are presented and compared to direct mechanics, along with a similar sensitivity analysis. Lastly, the whole bone stiffness estimated by orthotropic and isotropic QCT-based FE models are compared to experimental data in order to test the hypothesis presented in chapter one.

4.1 Map of Anisotropy

4.1.1 Trabecular Architecture

A vector plot was generated for a single frontal, sagittal, and transverse slice of the 3D map of anisotropy to visualize the principal stiffness orientation throughout the inner architecture (Figure 4.1). The vectors, represented by arrow glyphs, are aligned with the principal stiffness direction determined by direct mechanics for each HR-pQCT cube. For the seven specimens in this study, there was an average of approximately $13,387 \pm 751$ cube samples used to construct the map of anisotropy. When compared to a segmented HR-pQCT image of the proximal femur, the patterns of this map reflect all five principal stress lines visible within the trabecular bone. These stress lines are named as follows: (1) primary compression, (2) primary tension, (3) secondary compression, (4) secondary tension, and (5) greater trochanter stress lines. These lines are named
according to the principal stress they undergo during physiological loading configuration. There is a very clear representation of the primary compression line that connects the articulating surface of the femoral head during standing, to the medial cortical shaft. This is also the site with the densest trabecular bone. Second, we see a visible horizontal pattern connecting the medial surface of the femoral head to the superior neck. This is a particularly important structure that is commonly absent in previously published maps, and will play an important role in transverse loading. The patterns within the innermost neck and trochanter become somewhat less stable due to the very low bone volume in these regions. Nevertheless, there are visible arches that connect the medial and lateral surface of the cortical shell to the superior surface of the neck and greater trochanter. While the frontal plane is best for viewing the multiple structural patterns within the femoral trabecular architecture, realistic directionality was also observed in the sagittal and transverse planes as well (Figure 4.1). Only the results of a single specimen are reported here, however a representative slice from each specimen is contained in the Appendix.
Figure 4.1: Principal stress lines of trabecular bone (left) compared to HR-pQCT based map of anisotropy (right), in the frontal (top), sagittal (middle), and transverse planes (bottom). Arrows represent the principal direction of strength and are colored by principal stiffness.
Another diagnostic landmark we can use to qualitatively assess the architectural integrity of the different maps is called Ward’s triangle (Cummings et al., 1993), which is an observed shape in femoral radiography formed by the intersection of the primary and secondary compression lines with the primary tension line (Figure 4.5). Typically a healthy femur image will display a well-defined shape of Ward’s triangle, which will become less clear in an osteoporotic femur. The size of Ward’s triangle also alludes to the overall structural integrity of the trabecular architecture, where osteoporotic bones will present a triangle with larger area due to reduced architecture. Ward’s triangle can also be observed in the map of anisotropy for each specimen, with more or less definition depending on the bone mass within the femoral neck. This interspecimen similarity suggests that perhaps Ward’s triangle could provide a useful landmark for assigning directionality to all femur models.

Figure 4.2: Ward’s triangle represents the intersection of primary and secondary compression lines with the primary tension line (left). Example of Ward’s triangle within the map of anisotropy for a health femur specimen (right).
While the principal directions were the most important result of the map of anisotropy utilized in this thesis, the map also contains other useful data we can use to describe the architecture within the proximal femur. One example is the degree of anisotropy (DA) which describes the ratio between scalar stiffness in each orthotropic plane of symmetry. The DA can be visualized along with the principal directions using an ellipsoid glyph, where each major axis of the ellipsoid is oriented with the principal directions and scaled by the stiffness in that plane (Figure 4.3). This ellipsoid map describes a more complete picture of the anisotropy by showing the relative stiffness in the secondary and tertiary directions as well. As the HR-pQCT cubes became more isotropic in their apparent stiffness, the resulting ellipsoids become more spherical. Conversely, the ellipsoids of anisotropic cubes become very thin as the primary direction becomes stronger relative to the secondary and tertiary ones. This map reveals that the denser trabecular bone is consistently more isotropic than the low density bone. It also appears that trabecular bone is almost transversely isotropic in several regions within the femoral neck, where the DA between secondary and tertiary directions is small compared to the primary direction.
Figure 4.3: Map of anisotropy represented by ellipsoid glyphs, were each axis of the ellipsoid aligned with the principal directions and scaled by the stiffness in that direction, as calculated by direct mechanics.
4.1.2 Interpolation

The vectors describing the primary, secondary, and tertiary principal directions served as data points for the radial basis function (RBF) used to interpolate data from HR-pQCT images to the elements of QCT-based FE models. For each specimen a unique RBF was constructed using data from approximately $1 \times 10^4$ cubes, which was then used to interpolate principal directions to approximately $4 \times 10^5$ bone elements in the QCT-based model. Three examples of the directions interpolated to QCT elements are provided in Figure 4.5, for specimens with the maximum, median, and minimum T-score (-1.23, -3.50, and -5.83 respectively) (Figure 4.4). Each arrow in this figure represents the principal direction assigned to a single element within a QCT-based FE model. The blocks attached to the head and trochanter represent the PMMA caps. The directionality assigned to these elements also clearly exhibits the same patterns visible in the original HR-pQCT map of anisotropy, suggesting that the interpolated directions possess a high degree of architectural fidelity. Furthermore, despite the wide range in aBMD of the specimens, the patterns of internal architecture remain relatively fixed. This makes intuitive sense since the trabecular architecture of the proximal femur is largely shaped by the onset of bipedal locomotion in the developmental stage of life (Ryan and Krovitz, 2006). There is slightly higher variation visible in the low density specimen, but the same trabecular stress lines are still clearly observable.
Figure 4.4: Posterior half of interpolated maps of mechanical anisotropy, where each arrow glyph represents the principal direction interpolated for each QCT element. The internal patterns of architecture are visualized for the specimens with maximum (top), median (middle), and minimum (bottom) T-score to highlight similarities in directionality over a range of aBMD.

4.1.3 Directionality of Cortical Bone

Although the anisotropy measurements described in this thesis were designed only for analysis of trabecular bone, it is interesting to observe the principal directions determined for the cortical regions as well. When compared to previous studies that revealed the Harvesian system orientation using India ink (Baca et al., 2007), this anisotropy map shows good agreement in alignment in several cortical sites such as the femoral neck, shaft, and the greater and lesser
trochanters (Figure 4.5). The reason direct mechanics analysis is still able to determine
directionality in these regions lies in the automatically assigned boundary conditions, which are
unable to properly test directions perpendicular to the cortical surface, leaving only the directions
in-plane with the cortical shell. Numerically, this creates meaningless stiffness constants due to
the incomplete strain tests, yet the resulting rotation matrix still describes the orientation of
principal stiffness as parallel to the architecture of the cortical shell.
Figure 4.5: Osteon orientation adapted from Baca et al., 2007 with permission (left), compared to cortical element orientation determined by the interpolated map of anisotropy (right).
4.2 Sensitivity Analysis

The purpose of this sensitivity analysis was to determine the effect of segmentation threshold and cube size on the resulting principal directions calculated by direct mechanics. These parameters were tested because they introduce the most user bias to the methodology that is otherwise fully automated. The results of sensitivity analysis are presented as follows: a vector describing the principal stiffness direction was calculated for each cube, over a range of thresholds and cube sizes using direct mechanics. The vector corresponding to the threshold and cube size implemented within the final map of anisotropy (i.e. 150 mg HA/cm$^3$ and 5 mm respectively) was made the “default” orientation. The angular deviation was measured as the angle between the principal direction at each value and the “default” orientation. Thus the angular deviation at a threshold of 150 mg HA/cm$^3$, and cube size of 5 mm, was always zero.

4.2.1 Segmentation Threshold

The effect of segmentation threshold on angular deviation for each cube is illustrated in Figure 4.6. A visualization of the absolute changes in principal direction due to varied image threshold for a single cube from the head, neck, and trochanter is also provided in Figure 4.7. Increasing the segmentation threshold decreased the BV/TV of each cube, but the directionality was largely unaffected with only a few exceptions. In the low density areas in the neck and greater trochanter, higher thresholds resulted in perforated trabeculae causing visible deviations in principal directions. The region of smallest angular deviation lies between 120 and 180 mg HA/cm$^3$, suggesting that direct mechanics was highly insensitive the threshold values selected within this region. Thresholds above and below this range contain somewhat unpredictable changes principal directions.
Figure 4.6: Angular deviation between the principal direction of several segmentation thresholds and the “default” direction defined at a threshold of 150 mg HA/cm$^3$, measured at nine locations within the proximal femur.

Figure 4.7: Visualization of principal directions as the threshold is increased (left to right) for a single cube from the femoral head (top), neck (middle), and greater trochanter (bottom).
4.2.2 Cube Size

Increasing the cube size also had minimal effect on the principal directions in most cases (Figure 4.8). The angular deviation did gradually increase above the cube side length of 6 mm which represents effective averaging of principal directions as more architecture is included in the direct mechanics analysis. This gradual change can also be observed in a visualization of principal direction for different cube sizes (Figure 4.9). High variability in angular deviation was observed in cube sizes below 5 mm, which reflects the effects of direct mechanics applied to specimens with insufficient architecture to properly assess principal directions. The sudden angular deviation of the single trochanter specimen at large cube sizes can be explained by the inclusion of a new architectural feature such as cortical bone. This has a strong effect on the apparent direction of strength of a trabecular specimen due to the difference in density.
Figure 4.8: Angular deviation between the principal direction of several cube side lengths and the “default” direction defined at a side length of 5 mm, measured at nine locations within the proximal femur.

Figure 4.9: Visualization principal directions as the cube side length is increased (left to right) for a single cube from the femoral head (top), neck (middle), and greater trochanter (bottom).
4.3 Fabric Tensors

4.3.1 Trabecular Architecture

Anisotropy maps constructed using the MIL method to measure fabric anisotropy resulted in very similar directionality patterns as direct mechanics for both healthy and osteoporotic specimens (with T-scores of -1.23 and -5.83 respectively) (Figure 4.10). Again we see the primary compression line connecting the superior femoral head to the medial cortical bone, and primary tension line connection the medial femoral head to the superior neck. Patterns within the femoral shaft and trochanter are also visibly similar, suggesting that fabric anisotropy is very well aligned with principal stiffness, even in these low density areas. When the relative MIL magnitude was compared to the principal direction determined by direct mechanics, there was a visible difference in several regions, particularly around the marrow cavities. This could suggest that although the fabric and mechanical directions are well aligned, the magnitude of fabric may not present a realistic degree of anisotropy.
Figure 4.10: Mid-frontal slices of fabric and mechanical anisotropy maps using MIL method (left), and direct mechanics method (right), for a healthy (top) and osteoporotic specimen (bottom).
4.3.2 Cortical Architecture

This study found that volume fraction had minimal effect on angular deviation between the principal directions measured by either MIL or direct mechanics, suggesting that certain architectural scenarios are more likely to be responsible for any directional discrepancies. While the trabecular architecture patterns are too variable within the proximal femur to determine specific factors that cause this deviation, a qualitative analysis reveals that the majority of these errors arise in the transitional and cortical regions. The architecture becomes highly irregular in these regions, making it difficult to determine the apparent properties of small volumes. The directionality of a small sample of irregular cubes, from several anatomical sites, was measured using direct mechanics and MIL methods, and the principal directions were compared in a fixed frame of reference (Figure 4.11). Comparing the 3D orientation of the resulting arrows revealed that direct mechanics and MIL are well aligned in most instances except for curved, low density areas from the femoral neck. While this small data set is not conclusive, it does suggest that the eigenvectors of the fabric tensor are generally well aligned with the principal directions measured by direct mechanics, even in regions with low BV/TV and irregular geometry.
Figure 4.11: Directionality of cortical sample, 5 mm in length, from the greater trochanter (A), femoral neck (B,C), head (D), and low-density bone from the femoral neck (E), as determined by direct mechanics (center) and MIL methods (right).
4.3.3 Sensitivity Analysis

An identical sensitivity analysis was performed on the same cube subset using principal directions determined with the MIL method. Once again the principal direction of the chosen threshold (150 mg HA/cm³) and cube size (5 mm) were defined as the “default” orientation, and the angular deviation of each value was compared relative to this default. The results were analogous to the analysis of the direct mechanics method, where the directionality measured by the MIL method was not sensitive to the selected segmentation threshold, nor the selected cube size (Figure 4.12). There was also a similar increase in angular deviation at the lower cube sizes and thresholds. Interestingly, the extreme angular deviations observed in the neck and trochanter in the direct mechanics sensitivity analysis, are absent from the MIL measures. This suggests that the MIL method is less affected by trabecular perforation caused by over thresholding, and sudden architecture changes caused by large cube sizes.
Figure 4.12: Sensitivity analysis of cube threshold (top) and size (bottom) using MIL analysis to determine the principal directions using the eigenvectors of the fabric tensor. The “default” direction defined at a threshold of 150 mg HA/cm$^3$ and cube side length of 5 mm, measured at nine locations within the proximal femur.
4.4 Finite Element Analysis

4.4.1 Orthotropic and Isotropic QCT-based FE Models

Whole bone stiffness for each of the seven specimens was estimated by solving QCT-based FE models built with orthotropic and isotropic material properties, and loaded in a sideways fall configuration (Table 4.1). Linear regression was used to compare both isotropic and orthotropic model stiffness to experimentally derived stiffness obtained from previous study (Nishiyama et al., 2013). Coefficients of determination (R² values) were calculated from this linear regression (Figure 4.13). Orthotropic models showed no improvement in predicting experimental stiffness compared to isotropic models, with R² values of 0.780 and 0.787 for orthotropic and isotropic models respectively. The Fisher transform was used to convert Pearson correlation coefficients (r) into a z-value to test statistically significant differences between the correlations of isotropic and anisotropic models with experimental data. A two-tailed comparison resulted in a p-value of 0.98, indicating that there was no significant difference in correlation between model types.

Table 4.1: Results of solved QCT-based FEA for orthotropic and isotropic femur models.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>T-score</th>
<th>Number of Elements (x10³)</th>
<th>Solve Time (minutes)</th>
<th>Orthotropic Stiffness (N/mm)</th>
<th>Isotropic Stiffness (N/mm)</th>
<th>Experimental Stiffness (N/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1.23</td>
<td>1210</td>
<td>224</td>
<td>1894</td>
<td>2516</td>
<td>2323</td>
</tr>
<tr>
<td>2</td>
<td>-1.84</td>
<td>985</td>
<td>128</td>
<td>1553</td>
<td>2070</td>
<td>1658</td>
</tr>
<tr>
<td>3</td>
<td>-3.43</td>
<td>1056</td>
<td>147</td>
<td>1138</td>
<td>1626</td>
<td>1534</td>
</tr>
<tr>
<td>4</td>
<td>-5.83</td>
<td>842</td>
<td>262</td>
<td>612</td>
<td>828</td>
<td>872</td>
</tr>
<tr>
<td>5</td>
<td>-4.75</td>
<td>754</td>
<td>142</td>
<td>936</td>
<td>1283</td>
<td>1249</td>
</tr>
<tr>
<td>6</td>
<td>-3.50</td>
<td>891</td>
<td>145</td>
<td>1222</td>
<td>1790</td>
<td>1446</td>
</tr>
<tr>
<td>7</td>
<td>NA</td>
<td>1007</td>
<td>276</td>
<td>849</td>
<td>1230</td>
<td>1624</td>
</tr>
</tbody>
</table>
Despite their similar predictive capabilities, the orthotropic and isotropic models responded differently to the sideways fall loading. When compared to experimental data, the slope of model stiffness was greater than unity for isotropic models and less than unity for orthotropic models. Predictably, the orthotropic models were consistently weaker than their isotropic counterparts, which can largely be attributed to the reduced tissue stiffness in the transverse directions. The variance between models steadily increased as the specimens became stronger, suggesting that the cortical structures in particular were behaving differently, since
femoral strength largely determined by the mass and geometry in the cortical bone.

Visualizing the distribution of von Mises stress throughout the internal architecture reveals several structural patterns within both the orthotropic and isotropic FE models (Figure 4.14). The von Mises stress is a useful index in this context because it accounts for complex normal and shearing loads in three dimensions, including the additive effects of compressive and tensile stress in different planes. However, this index is always a positive scalar value and does not visually distinguish elements in compression and tension. The displacement applied to the trochanter PMMA cap resulted in high stresses distributed throughout the cortical bone in the femoral neck and shaft, and along the transverse trabecular structures within the femoral head and greater trochanter. The transverse trabecular structures within the femoral head effectively act as a buttress support for the superior neck by connecting with the medial surface of the femoral head. The cap on the femoral head was free to translate in the vertical plane, resulting in a tensile stress on the external surface of the medial shaft due to bending. Additionally, the vertical trabecular structure also supports the tensile load by buttressing the cortical bone within the inferior femoral neck.
Figure 4.14: von Mises equivalent stress field for orthotropic (left) and isotropic (right) models for the strongest (top) and weakest (bottom) femur specimens, after 1 mm compressive displacement was applied to the PMMA cap on the greater trochanter.
4.4.2 Cube Overlap

The final sensitivity analysis in this thesis constructed four different maps of anisotropy using different magnitudes of overlap between the cubes. This resulted in anisotropy maps with different degrees of detail due to the number of local anisotropy measurements. Four different QCT-based FE models were constructed using directionality interpolated from the different anisotropy maps. The reduction in overlap (i.e. sampling rate) of HR-pQCT cubes resulted in a maximum of 1.6% difference in whole bone stiffness of QCT-based FE models.

Table 4.2: Effect of different levels of HR-pQCT cube overlap (i.e. sampling rate) on QCT-based FE predictions of whole bone stiffness.

<table>
<thead>
<tr>
<th>Degree of overlap</th>
<th>Number of cubes</th>
<th>Whole bone stiffness (N/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 mm (50%)</td>
<td>12744</td>
<td>702</td>
</tr>
<tr>
<td>1.75 mm (33%)</td>
<td>5974</td>
<td>700</td>
</tr>
<tr>
<td>1.5 mm (15%)</td>
<td>3098</td>
<td>695</td>
</tr>
<tr>
<td>0 mm</td>
<td>1776</td>
<td>691</td>
</tr>
</tbody>
</table>

4.4.3 Fabric-based FEA

Finally, orthotropic models of the femurs with the highest and lowest aBMD were constructed with both fabric and mechanical maps of anisotropy. There was effectively no difference in predicted whole bone stiffness for either specimen (Table 4.3), suggesting that there is no practical difference in using fabric or mechanical based directionality in QCT-based FEA of the proximal femur.

111
Table 4.3: Differences in whole bone stiffness measured using principal directions by direct mechanics and MIL methods.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>T-score</th>
<th>Whole bone stiffness - direct mechanics (N/mm)</th>
<th>Whole bone stiffness – MIL (N/mm)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>-1.22</td>
<td>1894</td>
<td>1873</td>
<td>1.0%</td>
</tr>
<tr>
<td>Osteoporotic</td>
<td>-5.83</td>
<td>611.8</td>
<td>612.0</td>
<td>1.0%</td>
</tr>
</tbody>
</table>
CHAPTER FIVE: DISCUSSION AND CONCLUSION

This final chapter interprets the results presented in the previous chapter and summarizes the findings of this thesis. The methods and results of this work are compared and contrasted with that of similar studies. The limitations of the methods are reviewed, including the assumptions and simplifications used in image processing, mapping anisotropy, and QCT-based FEA. Possible directions for future study are also provided. The end of this chapter concludes the thesis by summarizing the original contributions of this study, and the implications for future research using image-based FEA to study bone health.

5.1 Overview of Findings

5.1.1 Mapping Anisotropy

A qualitative analysis of the map of anisotropy revealed that the principal directions measured by direct mechanics showed good agreement with the trabecular stress lines within the proximal femur. All the major compressive and tensile trabecular structures were observed within the map, and these patterns were found to be generally consistent between specimens. In addition, the alignment of principal directions within cortical bone elements showed good agreement with the orientation of the osteons. Based on observable congruence between the map of anisotropy and the visible microarchitecture, we can conclude that using numerous local measurements of anisotropy is an effective approach for quantifying the directionality of bone architecture within the proximal femur.

The mapping methodology presented in this thesis was highly automated, where the
segmentation threshold and cube size were the only parameters selected by the user. The sensitivity analysis demonstrated that segmentation threshold and cube size plays a minimal role in determining the principal directions, provided that the selected values fall within the certain bounds. Namely, these bounds are segmentation thresholds between 120-180 mg HA/cm$^3$ and cube sizes $\geq$5 mm. The precise threshold level for HR-pQCT segmentation plays very little role in characterizing directionality, as long as maximum trabecular connectivity is preserved. A cube side length of 5 mm was the smallest cube size that satisfies the continuum assumptions while avoiding any averaging of principal directions that occurs in larger measurement volumes. Thus, the low sensitivity of directionality to these user-selected parameters demonstrates the robustness of this mapping methodology. However, this conclusion may only apply to the human femur specimens analyzed in this study, and different image thresholds and cube sizes may be required for different scale problems such as smaller human bones, or even other species such as mice or elephant bones.

The registration and interpolation techniques utilized in this work also present a robust approach for linking material properties between different images of the same specimen. All the trabecular and cortical directionality observed in the HR-pQCT map of anisotropy was successfully translated to the elements of the QCT-based model. While these techniques were an effective approach for translating directionality, they were also one of the most time-consuming aspects of this methodology. For 3D rigid image registration, this was due to the manual calculation of coarse image adjustments prior to intensity-based optimization. Thus improving the automation of this component would greatly reduce the time commitment required by the user. For the RBF interpolation, the construction of RBF models from HR-pQCT data points was on the order of ten minutes, while the actual interpolation calculations for roughly $4\times10^5$ QCT
elements required approximately two hours per specimen. It is likely that interpolating directionality for every QCT element is unnecessary to capture the gradual changes in microarchitecture orientation. Thus the speed of interpolation could be improved by assigning directionality to blocks of elements, thereby reducing the number of calculations required. Overall, these results have demonstrated that the RBF interpolation and intensity-based registration algorithms implemented here provide a high degree of geometric accuracy at the cost of processing time.

5.1.2 QCT-based FEA

The material properties of the QCT-based FE models presented in this thesis have two fundamental components, the elastic constants describing the magnitude of stiffness, and the directionality describing the principal stiffness direction of the microarchitecture. Each element was assigned orthotropic elastic constants as a function of scalar density. The resulting stiffness matrix was then rotated to align with principal directions as a function of position within the proximal femur. The relationships used to calculate the elastic constants came from literature, while the directionality was interpolated from the map of anisotropy presented in this thesis. It is theoretically possible to also interpolate the elastic constants from the map of anisotropy, since they were calculated by the direct mechanics method in order to determine the principal directions. However, the HR-pQCT images used to construct this map are not available in vivo, therefore using elastic constants from this data would remove this work even further from clinical application. Furthermore, discarding the density distribution within the QCT image would have partially defeated the purpose of this work, which is to study the effect of anisotropic material properties on the accuracy of QCT-based FEA. To accomplish this task, this study
isolated the effects of directionality on model behavior by only interpolating the principal directions, and using QCT-based density to determine the elastic constants, since only QCT images can quantify density distribution of central skeletal sites in vivo.

When the whole bone stiffness predicted by QCT-based FE models was compared to experimental data, no improvement in accuracy was observed with the addition of orthotropic material properties oriented with the underlying microarchitecture. Since the directionality of the elements was well controlled in this study, we can conclude that the shortcoming in predictive accuracy is likely due to the magnitude of the elastic constants taken from literature. The nine orthotropic constants were based on studies that used experimental testing of numerous small trabecular specimens that were machined from whole bones. This suggests that the density-elasticity relationships derived from small scale samples do not translate well to whole bone models. The orthotropic relationships taken from literature describe a degree of anisotropy (DA) that gradually decreases as the BV/TV of each element increases. This behavior was also observed within the map of anisotropy presented in this study, although there was also considerable variance in DA depending on geometric location within the femur. This finding makes intuitive sense because the DA is directly determined by the organization of the microarchitecture, which changes depending on anatomical site. A location-dependent DA was not included by the material properties implemented in this study, and no such relationship currently exists in literature. Therefore future studies looking to incorporate accurate site-specific DA should consider a mapping methodology similar to the approach presented in this thesis.

The major trabecular structures within the proximal femur where highlighted when visualizing the von Mises stress distribution within the solved FE models. The highest stress was within the cortical shell in the superior and inferior neck. The trabecular structures effectively
buttress these regions by providing some perpendicular support, while simultaneously distributing loads over the cortical surface minimizing stress concentrations. The mass and geometry of the cortical bone is the largest contributor to whole bone stiffness and strength, which was significantly diminished in the weaker specimens. The trabecular architecture that supports these regions was also significantly reduced, which also contributes to the reduction in stiffness. The relative strength provided by the trabecular architecture relative to the cortical structure is still not fully understood within the proximal femur, and further study of the load sharing between these compartments is required.

The femur as a whole exhibits a certain degree of natural curvature that assists in withstanding the end-loads caused by body weight and muscular attachments forces. Under physiological loads, the curvature increases due to the compressive stress on the medial surface and tensile stress on the lateral surface. When a transverse load was applied, it was found that the femur was loaded against its natural curvature and thus a tensile stress was applied to the medial surface and compressive stress to the lateral surface. This also occurred within the trabecular structures as well, where the compressive and tensile stress lines became reversed. The shift in tensile and compressive loading directions throughout the femur suggests that the non-linearity of bone tissue stiffness may play a significant role the accuracy of whole bone stiffness estimated by QCT-based FE models. Thus the linear models implemented in this study may be insufficient for this particular loading scenario, and non-linear tissue stiffness should be a serious consideration for future studies of sideways falls on QCT-based FE models of the femur.

The last major finding of this thesis concerns the comparison of fabric-based directionality determined by the MIL method, to the principal directions measured by direct mechanics. The two methods demonstrated highly congruent directionality throughout the
architecture of both trabecular and cortical compartments. The application of MIL directionality to QCT-based FEA also resulted in no significant difference in whole bone stiffness estimates for both the strongest and weakest specimen, suggesting that the directionality they provide is interchangeable. Since experimental validation of the directionality is impossible for low density and irregular geometries, the direct mechanics analysis presented in this thesis is likely the closest mechanical validation possible for fabric-based measures in these regions. The direct mechanics approach to mapping anisotropy was much more time-consuming due to the multiple strain cases being solved in order to fully define the anisotropic stiffness matrix. Therefore if fabric anisotropy offers the same directionality, it may be the more computationally efficient approach. Where fabric and mechanical anisotropy measurements differ is the resulting degree of anisotropy, or ratio between scalars of the primary, secondary, and tertiary principal directions. The MIL is only a relative measure of the amount of architecture in any given direction, while direct mechanics actually determines the nine orthotropic elastic constants describing the apparent anisotropy. Thus mapping mechanical anisotropy may still be the superior approach for the purpose of calculating a location-specific DA within the proximal femur.

5.2 Synthesis

The two fundamental components of the material properties within each element of an orthotropic QCT-based FE model are the magnitude of the orthotropic elastic constants, and directionality of the orthotropic planes of symmetry. Previously published studies have approached these two components very differently, leaving it unclear what role the elastic constants and directionality play in FEA of the proximal femur. This ambiguity is increased by the lack of experimental validation for orthotropic models, where the only comparison being
made is relative to an identical isotropic model. This thesis attempted to approach this problem in two ways: by presenting a novel and highly realistic map of directionality, and by comparing both orthotropic and isotropic models to experimental data, which serves as a gold standard.

5.2.1 Density-Elasticity Relationships

Two approaches for determining the magnitude of the nine orthotropic elastic constants were found in literature, both of which express the magnitude as a power function of element density (i.e. $scalar \times density^{power}$). The first approach was the previously described regression methods by Yang and colleagues (1999), which derived relationships for each elastic constant as a function of volume fraction for 141 human cancellous cube specimens. Alternatively, modal analysis was also used to calculate the elastic constants that produce a certain natural frequency for the entire femur, which was validated using ultrasound transmission techniques (Taylor et al., 2002). This thesis chose the former approach for two reasons: the resulting values from modal analysis have not been validated using mechanical testing of whole bones, and secondly the relationships regressed from cube specimens were derived using a CT-based FEA approach similar to the methods presented here. When these orthotropic elasticity density relationships were compared to those studies using modal analysis (Peng et al., 2006; Baca et al., 2008; Yang et al., 2010), both the scalar and power functions resulted in higher Young’s moduli and lower Poison’s ratios. In other words, the material used to represent cancellous bone in this study was much stiffer and less deformable than material properties determined by modal analysis. For cortical bone, modal analysis had a greater power value in the density-elasticity relationship suggesting slightly more stiffness was attributed to cortical bone than in this study. Furthermore, modal analysis modeled cancellous bone a transversely orthotropic instead of fully orthotropic.
While this study observed a high degree of transverse orthotropy in certain regions such as the femoral head, this was not observed in low density regions in the neck and trochanter, particularly in regions of transition from trabecular to cortical architecture. When compared to another study that used similarly regressed density-elasticity relationships (San Antonio et al., 2012), the power values were identical for cortical and cancellous bone. The only major difference in elastic constants were the higher scalar values used in this study, but since the models are linear-elastic, this scalar value does not affect the measured correlation values.

Numerous studies have assigned different material properties for cancellous and cortical compartments, which is justified considering the different tissue organization in these structures. Nearly all the studies using orthotropic material properties, including this thesis, separated the two compartments based solely on the element density. Similar to other studies (Peng et al., 2006; Baca et al., 2008; Yang et al., 2010; San Antonio et al., 2012), this thesis assigned transversely isotropic tissue stiffness to elements with an apparent density >1.2 g/cm$^3$. The elements identified as cortical bone by this criteria were mostly limited to femoral shaft, since the thin cortical bone in the femoral head and diaphysis was difficult to distinguish from the trabecular region using density alone. Other studies did not report the geometric distribution of cortical bone within their models using this density threshold, and thus this research calls into question the accuracy of assigning material properties to cortical bone in this manner. QCT-based FEA could greatly benefit from separate analysis of the cortical and trabecular regions. Adjusting the cortical material properties may also improve correlations of model stiffness, and isolate the effect of trabecular anisotropy on the accuracy of model stiffness.

A density based division between compartments was difficult to establish at clinical resolutions, and it is likely that an image-based division is required for separate compartment
analysis and material assignment (Varghese et al., 2011; Treece et al., 2010). It is understood that straightforward cortical thickness measurement of clinical CT images become inaccurate below thicknesses of 2.5 mm (Hangartner and Gilsanz, 1996), while errors at the sub-millimeter cortices can exceed 100% (Prevrhal et al., 2003). This situation can easily occur in subchondral regions in the head of the femur, where the cortical thickness becomes very small relative to voxel size. Therefore more sophisticated segmentation techniques have been proposed using a mathematical model of the bone anatomy that is mapped to contours of the endosteal surface, producing thousands of independent thickness estimates per specimen that are accurate to 0.3mm (Treece et al., 2010). While this analysis was beyond the scope of this thesis, future work could apply this model to the contours obtained in this study, which would enable separate analysis of cancellous and cortical compartments. While it has been suggested that trabecular bone plays a lesser role in determining fracture in the proximal femur (Holzer et al., 2009), separate analysis of compartments could further explore this claim by determining the load sharing between the different structures.

5.2.2 Directionality

Nearly all the studies applying orthotropic material properties have measured the directionality of femoral architecture in different ways. The earliest attempts to quantitatively map directionality of the femur used machining and grinding of the lamellar structures in order to visualize the architecture orientation (Baca et al., 2008, Wirtz et al., 2003). Comparatively, the image-based FEA approach presented in this study provides a more detailed map of directionality that was also determined non-destructively. Furthermore this computational approach involved far less user bias compared to manually selecting directions based on the
observed architecture orientation. Another study only added directionality to regions where it could be safely assumed without measurement, such as the femoral neck and shaft, excluding other regions as too variable (Yang et al., 2010). The mapping approach presented in this thesis overcomes this limitation by using numerous local anisotropy measurements to capture the directionality patterns, even in highly complex areas such as the femoral head and low-density regions between the neck and greater trochanter. San Antonio and colleagues (2012) also used an FEA-based method to determine directionality, by simulating the stresses applied to the femur during gait and calculating the direction of principal stress element-by-element. Although unique in many respects, this method was not based on architecture like the approach proposed in this thesis, but instead used load-specific directionality and therefore is only applicable to the loading scenario used for its derivation (i.e. gait). Thus an architecture-based map of anisotropy has an advantage in this regard, by being applicable in any loading configuration.

A recent study by Marangalou and colleagues (2012) developed a similar map of anisotropy using numerous local anisotropy measurements. Directionality was quantified using a fabric tensor calculated using the MIL method on spherical volumes with 4 mm diameter, which is comparable in volume to the cubic volume assessed by direct mechanics in this study. A similar sensitivity analysis also confirmed that the directionality measured by MIL is not sensitive to the measurement volume, at least within a diameter ranging from 4 mm to 8 mm (Marangalou et al., 2012). The resulting directionality of this approach was highly similar to the map of anisotropy presented in this work, where all the trabecular stress lines were well represented. The only visible difference in directionality compared to the map of anisotropy presented here, is a lack of transverse structures visible within cancellous region of the medial femoral head. Whether this is a result of the mapping process or the specific specimen analyzed
is not clear due to the small sample size. Another methodology difference was the exclusion of cortical and transitional regions from MIL analysis. This thesis demonstrated that MIL was able calculate a reasonably realistic direction for cortical elements, but the study by Marangalou and colleagues (2012) did not provide rational for excluding MIL analysis of cortical bone, and assigned an isotropic stiffness to this compartment. Furthermore the criterion used to distinguish cortical and trabecular compartments was not discussed, and could not be compared to the density-based division used in this study.

5.2.3 Whole Bone Stiffness

Previous studies of orthotropic QCT-based FE models have only compared mechanical behavior to isotropic models of the same specimen. The reported results have been highly varied, with some studies claiming anisotropic material properties provide no change from isotropic models (Peng et al., 2006; Verhulp et al., 2006; Baca et al., 2008), while others claim significant strain differences in the femoral neck (Yang et al., 2010; San Antonio et al., 2012). Still, the accuracy of the models used in these studies is questionable due to the lack of validation with experimental data. This study appears to be one of the first attempts to address this gap, and it was found that orthotropic models with directionality aligned with the microarchitecture did not improve prediction of whole bone stiffness. While there is no analogous study to compare this finding with, smaller scale experimental studies have consistently demonstrated that the prediction of mechanical behavior improves when trabecular directionality is accounted for (Ulrich et al., 1999; Goldstein et al., 1993). These results suggest that variability explained by architecture directionality does not scale to the whole bone level, which has also been suggested by studies showing minimal differences between orthotropic and isotropic material properties.
(Verhulp et al., 2006; Peng et al., 2006; Baca et al., 2008). However, the results of this thesis do not fully agree with this conclusion since the different material properties produced visibly different mechanical behavior despite the lack of improvement in stiffness prediction. Thus these findings are closer in agreement with other studies that found different strain behavior within the neck of orthotropic models (San Antonio et al., 2012; Yang et al., 2010).

Marangalou and colleagues (2012) used FEA of a segmented micro-CT image of the entire proximal femur as a gold standard to compare different material models. With a voxel size of 80 µm, this micro-CT-based FE model included all the microarchitecture responsible for anisotropic material properties, providing the most realistic FE model possible. While a micro-CT gold standard is an appealing concept, comparing one numerical model to another is only useful if one has been thoroughly validated. Thus experimental data still forms the bedrock of validation studies, but with a sufficient sample size, developing a fully validated micro-CT based FE model of the proximal femur is certainly within reach. Another advantage of comparison with experiments is the highly controlled boundary conditions, which in this study refers to the femur orientation and PMMA caps which were included in the FE model for consistency. Other studies that neglected to use mechanical validation, applied loads directly to the bony surfaces as either point loads (Marangalou et al., 2012; Verhulp, 2006) or distributed loads (Peng et al., 2006). This introduces considerable interstudy variability since the location, distribution, orientation, and magnitude of these loads become somewhat arbitrary without a fixed reference, such as an experimental design.
5.3 Limitations

5.3.1 Image Processing

The methodology proposed in this study is still in its proof of concept stage and has several limitations that must be discussed. As previously mentioned, the segmentation of HR-pQCT data used a simple filter and threshold process with a manually selected threshold value to preserve maximum trabecular connectivity, likely resulting in an overestimation of total bone volume. While it was shown that the selected threshold had little effect on the measured orientation of architecture, the robustness of this approach could be further improved by increasing the automation of femur segmentation and removing any user bias. This could be done using an adaptive threshold algorithm that selects a threshold that minimizes the change in BV/TV (Pistoia et al., 2001). Dual thresholding could also optimize segmentation by analyzing trabecular and cortical compartments separately (Buie et al., 2007), which would also remove the interference of cortical bone in direct mechanics analysis.

The image registration method used in this study was very robust, and was able to match femur geometry for all specimens, but the process was slow. The user was required to the majority of the coarse transformations to ensure that the registration algorithm only dealt with small angle adjustments. A possible improvement could determine the principal axes of both images and align them for an automatic estimation of the coarse image adjustments (Alpert et al., 1990). Combined with automated superior surface alignment, this could significantly reduce user interaction and speed up the registration process. Alternative software also uses point-based registration, which aligns user defined points on the surface of the bone. While this introduces some variability due to manually assigning the points on different images, the femur has several useful landmarks for this type of analysis, such as the lesser and greater trochanter.
5.3.2 Mapping Anisotropy

This study did not distinguish trabecular and cortical bone cubes, and as a result the direct mechanics analysis of partial or cortical cubes was a frequent concern. Six uniaxial strains are required to fully describe mechanical anisotropy of a particular specimen, which requires a reasonably cubic geometry in order to apply the proper boundary conditions. For curved cortical surfaces, or irregular trabecular architecture in low density areas, the partial cubes may leave one or two dimensions unable to strain properly, which results in either two or four improper strain cases within direct mechanics. When the orthotropic planes of symmetry are assessed for these incomplete cubes, the magnitude of the secondary, tertiary, and sometimes even the primary stiffness can become meaningless. Nevertheless, the primary direction of strength that represents the orientation of microarchitecture remained realistic, since the only viable testing directions occurred along where the strains were applied successfully. For this reason we felt confident including the directionality provided by measurements of partial cubes in the final map of anisotropy, since ultimately only the principal directions were interpolated from this map into elements of the QCT-based FE model.

The mathematical density-elasticity relationships determined by Yang and colleagues (1999) formed the basis of the material table used in this study. These constants were derived by direct mechanics analysis of a large database of vertebral trabecular specimens, and describe a degree of anisotropy that decreases with larger bone volume fraction. While this study had similar findings for the degree of anisotropy, it may also be possible to interpolate femur-specific anisotropy ratios from the direct mechanics analysis performed in this study. However, the aforementioned issues with direct mechanics analysis of cortical and partial cubes would need to be addressed, as the degree of anisotropy depends on the scalar stiffness of the secondary and
tertiary directions, which are confounded by incomplete cubes. A possible solution would be to separate the trabecular and cortical structures during image processing, prior to anisotropy measurements. Previous studies mapping fabric anisotropy of the proximal femur typically exclude all cortical bone and simply use the identity matrix to represent the fabric tensor in these cases (Marangalou et al., 2012). This may not be the best approach as even the cortical bone possesses some degree of directionality (Baca et al., 2007), and is well understood to be transversely isotropic in nature (Dong and Guo, 2004).

5.3.3 Finite Element Analysis

The custom in-house finite element solver used in this study (FAIM v6.0, Numerics88), required some alterations to incorporate orthotropic material properties for large-scale whole bone models. The software was modified to store a custom local stiffness matrix for each element within the entire model. This matrix can be constructed using isotropic or orthotropic constants, but the orthotropic constants remained oriented with the specimen coordinate system, and could not be locally rotated. Other commercial solvers such as ANSYS have the capability of rotating the local coordinate system of each element, but require much more memory to solve large-scale models, which is detrimental for the complex material tables being implemented in this project. As a result, this study had to construct a stiffness matrix for each element, using orthotropic constants derived from the element density, and rotate the stiffness matrix to align the orthotropic planes with the principal directions interpolated from HR-pQCT data. The major limitation here is that FAIM’s current architecture only accepts orthotropic constants, which cannot be directly calculated from the rotated stiffness matrix using the same analytical formulae. However, an approximation can be made by extracting the Young’s and shear modulus
values from the rotated matrix as if it were in orthotropic orientation, but this cannot be applied for Poisson’s ratio, and this value was fixed at 0.3 for all elements, which is common practice in even within the most recent orthotropic modeling studies (Marangalou et al., 2012; Yang et al., 2010). While this assumption does not fully obey the laws of an orthotropic material, it is a sufficient approximation to compare differences between isotropic and orthotropic femur models, until FAIM can be modified to accept fully anisotropic stiffness matrices for each element.

Finally, the models in this study used a linear-elastic tissue modulus that does not reflect the asymmetric effects of tensile/compressive stress in bone tissue. Since the loading configuration presented here approximates three-point bending, we can assume that significant tensile stress is present in the medial cortical shell. Considering that this is also the location of fracture seen during experimentation of these specimens, the assumption of symmetric tensile/compressive tissue stiffness may need to be reexamined. While predicting failure is outside the scope of this thesis, the Tsai-Wu failure criterion could be implemented, which models the tension-compression nonlinearity in a single equation (Tsai and Wu, 1971). More recently, a fabric-dependent Tsai-Wu failure criterion that accounts for trabecular anisotropy was proposed (Cowin, 1986), and adapted for high resolution vertebrae models (Wolfram et al., 2012). Although fabric tensors form the basis for anisotropy included in these advanced yield criterions, the map of mechanical anisotropy presented in this study could also be as the basis for directionality implemented in this failure analysis.
5.4 Future Directions

5.4.1 Improved Material Properties

From the results presented in this thesis, it is clear that additional work is needed to further investigate the material properties of QCT-based FE models of the proximal femur. While the directionality is now far better understood, a more sophisticated method for assigning elastic constants and DA to each element is still required. The compressive tissue stiffness has been thoroughly investigated with small tissue samples, yet attempts to apply these relationships to whole bone models have only achieved limited success. The best approach to solving this problem is likely similar to the approach by Austman and colleagues (2009), by developing a custom density-modulus relationship based on mechanical testing of numerous whole bone specimens. Mechanical testing protocols for femur specimens have become reasonably consistent in scientific literature, thus a custom mathematical relationship for the tissue modulus of the proximal femur could be achieved with a sufficiently large sample size. In addition to the scalar tissue modulus, the DA must be better characterized in order to accurately predict the stiffness ratio between principal directions for each element. Conceivably, this could be accomplished using a large scale direct mechanics analysis similar to the methods presented in this study, since the DA of trabecular bone can only be directly calculated from high resolution data. Thus it would need to be mapped to a QCT model in the same way as the directionality was mapped in this study, which would have the advantage of being highly location-specific.

Before this analysis can be performed, it is also clear that the trabecular and cortical structures need to be separated. The majority of studies attempt to distinguish the compartments based on element density, but at clinical resolutions this approach is highly unreliable. Therefore, an image-based separation of compartments is recommended, since reasonably accurate methods
for the proximal femur have recently been published (Treece et al., 2010). Differentiating cortical and trabecular bone is highly appealing from a modeling perspective since the structural organization and mechanical behavior of these tissues are very different. Furthermore, the direct mechanics analysis of the DA only applies to trabecular bone. Fortunately the transverse isotropy of cortical bone has already been well studied (Dong and Guo, 2004).

5.4.2 Failure Load Prediction

Whole bone stiffness is a useful parameter for comparing model behavior with different material properties, but ultimately the parameter we are most concerned about is the failure load of bone, which corresponds to fracture. Several image-based FEA studies have achieved reasonably accurate failure load predictions compared to experimental data, but this has not yet been attempted in the femur with anisotropic material properties. Several methods exist in literature for calculating failure loads using linear (Nishiyama et al., 2013; Keyak et al., 1998) step-wise linear (Dragomir-Daescu et al., 2011), and bilinear elasto-plastic models (Koivumäki et al., 2012). A non-linear, elasto-plastic method is likely the most realistic approach for predicting failure of whole bone models, however the crux comes when selecting the correct tissue yield stress or stain. This is also where anisotropy plays the largest role, as the yielding of bone tissue is highly direction dependent. Fully anisotropic tissue yielding has been proposed in the fabric-based Tsai-Wu criterion, which incorporates the directionality of trabecular bone to estimate surface yield properties (Wolfram et al., 2012). This particular method has not been applied to QCT-based FEA of femurs, and so far only bilinear models have attempted to account for differences in yielding due to tension and compression in isotropic models (Koivumäki et al., 2012). While computationally efficient elasto-plastic modeling software is still being developed,
an accurate non-linear tissue yield criteria must still be determined specifically for orthotropic FE models and experimentally validated.

5.4.3 Potential for Clinical Application

Orthotropic FE models are still in the proof of concept stage, and in order to move these methods towards a clinical application two primary concerns need to be addressed. The first is the computation time required to build and solve the orthotropic models from clinical imaging data. Assigning every element its own stiffness and orientation resulted in very large models, requiring around 20 GB of memory to solve on 4 CPUs. Reducing the stiffness values to 8-bit data (Nishiyama et al., 2013) and binning the principal directions (Baca et al., 2008), could reduce the number of materials by a more than a factor of 10. While the solving speed of FAIM was acceptable considering the size of the models, this could also be improved by increasing element size. Thus the effect of element size on orthotropic model accuracy also requires thorough investigation.

The second and most important limitation of orthotropic models, from a clinical application perspective, is the lack of patient-specific anisotropy maps. The architecture-based maps that provide the best representation of structural directionality are derived from high resolution images that are not obtainable in vivo. A method for mapping anisotropy of gray-scale QCT data has been proposed using weighted averages (Tabor et al., 2007), but the accuracy and robustness of this method has not yet been fully determined. The only alternative to QCT-based methods is a generalized map of anisotropy that would be interpolated to a patient-specific model of the femur. This could take the form of a medical atlas, which is an average model constructed from a large data base of scans. Medical atlases are used in radiology of the brain, which
involves mapping a patient-specific image to a generalized image from a large number of subjects which represents “normal”. An average map of anisotropy may be possible since the major structures (i.e. trabecular stress lines) observed in this study were consistent across several specimens over a range of aBMD values. Using deformable registration, a generalized map of anisotropy could be interpolated to the unique geometry of each patient. Assuming that an average model with sufficient convergence can be achieved, this approach would combine an approximated directionality with patient-specific density and stiffness values to provide more realistic material properties.

5.5 Conclusion

This study has made four original contributions to the field of image-based FEA of bone. First, a novel methodology for quantitatively mapping anisotropy of complex microarchitecture was proposed and applied to HR-pQCT images of the proximal femur. Second, a robust approach for mapping location-specific material properties from high resolution images to FE models of clinical QCT images was developed. Third, it was revealed that orthotropic QCT-based FE models, with directionality aligned with the microarchitecture, were equally predictive of whole bone stiffness compared to isotropic models. Lastly, fabric-based directionality was shown to be well aligned with principal directions measured by direct mechanics throughout the proximal femur, with no effective difference in whole bone stiffness for a healthy and osteoporotic specimen. The primary advantages unique to the methodology of this thesis included a more robust architecture-based map of anisotropy with a high degree of directional detail, and an experimental gold standard to compare different material models.
The results of this study lead to the rejection of the proposed hypothesis that orthotropic material properties aligned with microarchitecture improve the accuracy of QCT-based FE models compared to isotropic material properties. However, this conclusion only extends to the methods used in this study, that is to say that applying rotated stiffness matrices to 0.625mm hexahedral elements does not sufficiently capture the factors responsible for variability in whole bone strength. It is very likely that the degree of anisotropy used in this study was overly simplistic, since it was derived from a highly homogeneous set of vertebral trabecular specimens. The ratio of scalar stiffness between the different principal directions is determined by microarchitecture, and will therefore change throughout the geometry of the proximal femur. Therefore accurate quantification of the DA will require a map of anisotropy similar to the methodology presented in this thesis.

The sample size presented in this work is too small to definitively prove that orthotropic material properties do not improve model accuracy, but it does question how much variability in whole bone stiffness is explained by anisotropic material properties. The directionality of orthotropic material properties was well controlled in this study, therefore our next line of inquiry should focus on the magnitude of the orthotropic elastic constants, which up until now, have not been validated in whole bone models with experimental data. This thesis can make three clear recommendations in this regard. First, the cortical and trabecular regions need to be analyzed separately, and that an image-based method for separating these compartments is likely the best approach. Second, a femur-specific relationship for the elastic constants must be determined using experimental data of whole bone testing. Lastly, all future studies using orthotropic material properties should also attempt to control the directionality, and stay aligned with the underlying microarchitecture as much as possible. While the direct mechanics approach
presented in this thesis may be too complex for other studies, it was shown that directionality determined from fabric-based anisotropy measures, such as the mean intercept length, are equally accurate while less computationally intensive.

QCT-based FEA is becoming a promising clinical tool for non-destructive estimation of patient-specific bone strength *in vivo*. The accuracy of these FE models depends on realistic material properties that are able to detect changes in stiffness and architecture that occur in degenerative bone diseases such as osteoporosis. The material properties for these models are determined by the scalar density and directionality of the microarchitecture present within each element. For the most part, the directionality of the microarchitecture has been ignored in studies using QCT-based FEA, therefore the research presented in this thesis is an important check of this structural assumption. In other words, we need to understand the effects of anisotropic material properties before we can choose to ignore them. Efforts thus far in orthotropic FE modeling are still in the proof of concept stage, and while the limited evidence suggests that incorporating anisotropic material properties do not improve model accuracy, further study is still needed to confirm this finding before clinical application of QCT-based FEA can confidently proceed without them.
BIBLIOGRAPHY


Treece, G.M. Gee, A.H. Mayhew, P.M. Poole, K.E.S. 2010. High resolution cortical thickness measurement from clinical CT data. Medical Image Analysis 14: 276-290.


Yang, H. Ma, X. Guo, T. 2010. Some factors that affect the comparison between isotropic and orthotropic inhomogeneous finite element material models of femur. Medical Engineering and Physics 32, 553-560.


APPENDIX A: Additional Maps of Anisotropy

This appendix provides visualizations of the mid-frontal slice of the map of anisotropy and a clipped view of the segmented HR-pQCT for each specimen used in this study.

**Specimen 1**: ISQ C0009478, Mechval_id 05
Specimen 2: ISQ C0009482, Mechval_id 06
Specimen 3: ISQ C0009536, Mechval_id 10
Specimen 4: ISQ C0009484, Mechval_id 14
Specimen 5: ISQ C0009486, Mechval_id 15
Specimen 6: ISQ C0009604, Mechval_id 34
Specimen 7: ISQ C0009606, Mechval_id 35
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