Animal experiment (rabbit) to demonstrate changes in trabecular bone mechanical properties over time using finite element analysis.

Shuo Yang 1974-

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ANIMAL EXPERIMENT (RABBIT) TO DEMONSTRATE CHANGES IN TRABECULAR BONE MECHANICAL PROPERTIES OVER TIME USING FINITE ELEMENT ANALYSIS

By
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B.S. Beijing Jiaotong University, 1997
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Mechanical Engineering Department
Orthopaedic bioengineering Laboratory
University of Louisville
Louisville, Kentucky

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September 28th, 2006

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ABSTRACT

ANIMAL EXPERIMENT (RABBIT) TO DEMONSTRATE CHANGES IN TRABECULAR BONE MECHANICAL PROPERTIES OVER TIME USING FINITE ELEMENT ANALYSIS

Shuo Yang

September 28th 2006

Decreased strength of trabecular bone is a direct effect of osteoporosis, which can be evaluated by finite element analysis. However, computational limitations have restricted previous trabecular bone analyses primarily to the linear domain. In addition, previous work was largely invasive and the corresponding finite element models were typically homogeneous.

Nonlinear heterogeneous finite element analysis was used to calculate trabecular bone apparent strength directly from in vivo micro computational tomography (micro-CT) scans. Through a series of validation experiments, it was shown that this nonlinear modeling is more accurate in evaluating trabecular bone mechanics in osteoporosis than previous work. A parameter driven set of material properties was employed in the finite element models using gray levels in the form of Hounsfield units as the independent variable. This enabled the finite element models to capture the variations of material properties of trabecular bone. The methods and techniques for converting the micro-CT scans into finite element models, defining the finite element models (element type,
material properties characteristics) and solving the models are discussed in detail. In addition, the techniques developed for image preprocessing, such as image registration and image degradation, are also provided in this dissertation.

The scanning, image processing and modeling methods and techniques were applied to two groups of rabbits, an ovariectomy group and a control group, to evaluate the time-course of trabecular bone osteoporosis. Our experiment showed that ovariectomy significantly slows the normal bone strength increase over time observed in the control group. The strength increase over time was due to a combination of increased bone architecture indices such as volume fraction and trabecular thickness as well as increased material properties due to greater bone tissue density. Compared to heterogeneous models, the homogeneous models reflected less strength increase over time because they lack the capability to capture tissue level material property variations. Volume fraction analysis alone resulted in even lower predicted increases in bone strength because it could only monitor the bone apparent level density variation. Thus the nonlinear heterogeneous models, with parameter driven material definitions, are more accurate than other types of models or methods.
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CHAPTER 1. INTRODUCTION

Healthy bone is critical for the human skeleton to function well. However, some bone metabolic disorder, such as osteoporosis, will severely decrease bone strength and final result in fracture. Osteoporosis evaluation is important in predicting or healing this disorder. Image analysis and mechanical experiment are both very commonly used methods. Besides, the computational biomechanics method, which using micro-CT technique and finite element analysis (FEA) together, becomes more promising. In this dissertation, a heterogeneous nonlinear finite element analysis method will be introduced to evaluate rabbit bone osteoporosis.

1.1. Trabecular bone

A major component of the human musculoskeletal system is bone, which supports our body weight, facilitates our body motion, protects our organs, and also plays critical roles in mineral homeostasis and production of various blood cells. Structurally, bone is divided into two categories, cortical and cancellous (Figure 1.1.1). Approximately 75% of the adult human skeletal mass is cortical bone, which forms the outer wall of all bones and is largely responsible for a supportive and protective function. The remaining 25% of the bone mass is cancellous bone, which is also referred as trabecular bone. ‘Trabecular’ comes from a Latin word "trabs" meaning beams or timbers. As described by the word, trabecular bone is made up of networks of tiny strands of bone called trabeculae which
are either plate-like or rod-like or the combination of both. Trabecular bone plays very important roles in load transmission and energy absorption in major joints such as the knee, the hip and the spine (Silva et al., 1997). At the ends of long bones, i.e. the femur in the leg or the radius in the arm, the cortical bone becomes thinner and the bone is predominantly made up of trabecular bone. This predominance of trabecular bone compared to cortical bone is also found in the vertebrae, where more than 75% of the load is carried by trabecular bone (Silva et al., 1994).

![Figure 1.1.1. Bone Structure: trabecular bone and cortical bone.](http://www.medes.fr/Eristo/Images/BonPhysiologySmall.gif)

**1.2. Significance of the research on trabecular bone**

Trabecular bone is the most metabolically active type of bone. Although it accounts for only 25% of the skeleton by weight, it accounts for 75% of bone remodeling surface area (Patel et al. 1993). During normal remodeling, osteoclasts (bone-resorbing cells) excavate small cavities in the bone, which are subsequently refilled by osteoblasts (bone-forming cells) (Jee 2001). This homeostasis of bone resorbing and forming activities
maintains healthy trabecular bone. Due to osteoporosis, a common condition among elderly or post-menopausal women, the volume fraction (BV/TV, calculated by dividing the bone material volume (BV) by the total sample volume(TV)) of the porous structure can decrease. As shown in Figure 1.2.1, compared with normal trabecular bone, osteoporotic trabecular bone is more porous with rod-like trabeculae getting thinner and plate-like trabeculae getting penetrated and degraded to rod-like trabeculae. This results in decreased bone strength and increased bone fracture risk.

Osteoporosis is a major disease. The National Osteoporosis Foundation in the USA reported that by 2010, about 12 million people over the age of 50 are expected to have osteoporosis. By 2020, 14 million cases of osteoporosis are expected (NOF 2002). This increase will cause the number of hip fractures to double or triple by 2040 (Schneider and Guralnik 1990). Another report (Ray et al. 1997) showed that the annual direct medical cost in 1995 totaled $13.8 million (or $17.5 million adjusted to 2002 dollars) for the treatment of osteoporotic fractures in the USA (hip fractures alone were responsible for 63% of the total,). This exceeds the annual expenditures for breast and gynecologic malignancies combined (Hoerger et al. 1999). Thus, research on the pathology of osteoporosis, fracture healing and related osteoporosis assessing methodology has the potential to improve human life and reduce life expenses.
1.3. Methodology in structural analysis of trabecular bone

Decreased strength is a direct effect of osteoporosis, which causes bone to be at greater risk of fracture. Because of the spongy structure, trabecular bone strength depends not only on the trabecular tissue properties, but also the micro-structure or spatial arrangement of the trabecular bone.

To avoid ambiguity, trabecular bone properties, morphological or mechanical, are divided into two categories: tissue level properties and apparent level properties. Tissue properties refer to the properties evaluated at the trabeculae level while the apparent level properties are at the overall level of a trabecular bone specimen as shown in Figure 1.3.1.
At the apparent level, trabecular bone is a heterogeneous, porous, anisotropic biological material. The compressive stress-strain curve of trabecular bone shows two regimes of behavior (Figure 1.3.2). The small strain, linear-elastic response of trabecular bone results from the elastic bending and compression of the trabeculae (Bayraktar et al., 2004a; Rietbergen et al., 1995). The linear-elastic regime ends when the trabeculae begin to collapse. For low density trabecular bone, elastic buckling of the trabeculae may be responsible for collapse. For high density trabecular bone, plastic yielding or damage may be the reason (Bayraktar et al., 2004a and 2004b). Progressive collapse of trabeculae leads to the long, horizontal plateau of the stress-strain curve (Figure 1.3.2).
Mechanical testing is the most direct way to evaluate trabecular bone apparent mechanical properties. Most of the methods for testing traditional materials, such as ductile metals, also apply to trabecular bone. Tensile testing is one of the most accurate methods for measuring bone properties, provided that force is applied without inducing a coupled bending moment (Keaveny et al., 1994; Reilly et al. 1974). Compressive testing of bone specimens allows the use of relatively small specimens (Turner and Burr, 1993). Torsion testing has been used to study trabecular bone mechanical properties in shear (Turner, C.H., Burr, D.B., 1993). Indentation testing was used for measuring trabecular cube Young’s modulus and strength (Hvid and Hansen 1985; Sumner et al., 1994). The mechanical experiment is very important when examining the trabecular bone mechanical properties. Yet the invasive or destructive characteristic of these methods prevent the application in some circumstances; this is especially true for in-vivo studies.

Trabecular bone morphology study, also known as trabecular architecture study, is a non-mechanical approach in studying trabecular bone. In these studies, a list of morphological parameters (bone indices) is calculated and the results related to bone quality because of historical correlation. The commonly used indices are shown in Table 1.3.1.

<table>
<thead>
<tr>
<th>Terminology</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone volume fraction</td>
<td>BV/TV (denote as ( P_F ))</td>
</tr>
<tr>
<td>Trabecular plate number</td>
<td>Tb.N (denote as ( P_L ))</td>
</tr>
<tr>
<td>Trabecular plate thickness</td>
<td>Tb.Th=( \frac{P_F}{P_L} )</td>
</tr>
<tr>
<td>Trabecular plate separation</td>
<td>Tb.Sp=(1−( P_F ))/( P_L )</td>
</tr>
<tr>
<td>Surface-to-volume ratio of bone</td>
<td>BS/BV=2×( P_L )/( P_F )</td>
</tr>
</tbody>
</table>
These indices are used individually or jointly to predict trabecular bone mechanical properties. Goulet and Pothuaud found that using bone volume fraction with mean intercept length or other topological parameters explained up to 90% of the variation in trabecular bone modulus and strength (Goulet et al., 1994; Pothuaud et al., 2002).

However, using morphology indices to predict trabecular bone mechanical properties involves complex calibration work and the calibrated relations may be subject to change from sample to sample or group to group. In addition, morphology study could not fully explain the variation of trabecular bone apparent stiffness and strength because the mechanical properties depend not only on bone architecture but also bone mineralization quality which cannot be quantified by bone structural indices. The effects of architectural and mineralized tissue qualities on specimens cannot be discriminated. Owing to the development of high-resolution imaging systems (primary CT and MRI) and computing capability, this requirement can be accomplished by computer simulations with micro finite element analysis (micro FEA), which includes both the complete three-dimensional architecture along with necessary tissue property information to predict response under a simulated loading condition.

1.4. Background of FEA in the trabecular bone study

The FE method was applied to trabecular bone study in the early 1970s. McElhaney et al. (1970) developed a porous block model of trabecular bone based on integration of spring stiffness loaded in parallel or in series. Pugh et al. (1973) modeled the subchondral trabecular bone as collection of structural plates and concluded that bending and buckling were major modes of deformation of the trabeculae. Williams and Lewis (1982) modeled
the structure of trabecular bone with plane strain finite elements to predict the apparent transversely isotropic elastic constants.

From 1985, 3D models were used to model the trabecular structure. The common technique at that time was simplifying the trabecular structure to different cell elements based on the trabecular characteristics (rod-like or plate-like) (Gibson 1985; Beaupré and Hayes, 1985; Hollister et al., 1991). With micro imaging techniques (primary micro-CT and serial sectioning) brought into use in the late 1980s, more detailed, and thus more accurate, trabecular finite element models were constructed and solved. Feldkamp et al. (1989) used micro-CT scanning, with a typical voxel size of 50 µm, to represent trabecular bone structure. The 50 µm voxel size is representing a cube of 50×50×50 µm dimensions; this is the same as having image slices with 50×50 µm pixel resolution and 50 µm between slices. Hollister and Kikuchi (1992), Hollister et al. (1992), Fyhrie et al. (1992), Edidin et al. (1993) and Fyhrie and Hamid (1993) used voxel data sets to construct detailed FE models of small cubes up to 2.3 mm in dimension.

From the early 1990s, with the increase of computation capability, finite element analysis has been used widely and intensively in the trabecular bone mechanical property studies. The flourish of this technique in biomechanics area is partly owed to the availability of commercial FE software and auxiliary techniques in trabecular bone modeling such as efficient linear FE solvers (Van Rietbergen et al., 1996), accurate meshing techniques, etc. However, computation capability limits the trabecular bone analysis primarily to the linear domain (i.e. well before failure can occur). Instead of solving directly for the apparent strength using nonlinear FE modeling, one category of studies in the literature uses the apparent stiffness to predict the apparent strength based
on the fact that the bone apparent strength is highly correlated to the apparent stiffness (Fyhrie and Vashishth, 2000; Rho et al., 1995; Yeni and Fyhrie 2001). Another category of research evaluates bone apparent strength by linear analysis through counting the number of elements having strains exceeding the yield strain (Pistoia et al., 2002, Niebur et al. 2001). The latter technique was reported to greatly overestimate the apparent yield strain of the overall bone.

Currently, with properly developed techniques, directly solving the trabecular bone sample strength using nonlinear FE models is possible. This approach was shown to be more accurate than the two methods mentioned above.

1.5. Task of the research

There are two factors that affect the bone strength over time. One is caused by the fatigue process, in which micro-cracks will propagate through the bone material and cause a gross fracture of the bone as a whole. The other factor is the remodeling process or defects in the remodeling process (Huiskes and Kaastad, 2000a). The latter is known to be age related and is typically seen in post-menopausal women. If finite element models could be constructed on the same region of interest (ROI) from in vivo scans that are taken at two or more times during a period of time, it is possible to non-invasively evaluate the bone mechanical properties (stiffness and strength) variation during this period of time. If the technique is proved to be accurate enough, then finite element analysis could be employed to predict or quantify the trabecular bone osteoporosis and fracture risk. It could also be used to evaluate the fracture healing treatment or improvements in bone quality due to treatment for osteoporosis.
Thus, the primary purpose of the present study is to develop a robust routine and techniques to evaluate the capability of using finite element models to quantify the changing trabecular bone mechanical properties, (i.e. stiffness and strength), at different time points. The secondary purpose is to evaluate the accuracy of \textit{in vivo} finite element models on trabecular bone, which is the prerequisite for the first purpose. To accomplish these two goals, the following sub-goals are addressed.

I. Develop an image registration method which can register scans made on the same region of interest (ROI) of a living animal at different times. This technique guarantees that the finite element analysis is performed on exactly the same ROI of the animal.

II. Develop necessary image preprocessing techniques, such as image degradation, unconnected element elimination and image segmentation to prepare the scans for modeling.

III. Develop a fully automatic technique to convert the image stacks into heterogeneous finite element models and generate ABAQUS input file with proper material definition.

IV. Develop a technique for representing the model heterogeneities and calibrating the trabecular tissue stiffness and tissue strength for \textit{in vivo} finite element models.

V. Develop methods that can solve the large scale nonlinear models both accurately and efficiently.
CHAPTER 2. PRELIMINARY STUDIES

Before converting the CT scans into finite element models, considerable work must be done on the scans to guarantee the accuracy and efficiency of any subsequent finite element analyses. This chapter begins by introducing the difference between micro-CT and clinical CT, then systematically introduces the techniques used for registering micro-CT scans taken at different time points as well as the techniques for validating the registration process. Some image preprocessing methods are described such as image degradation, image segmentation and unconnected voxel elimination. At the end of the chapter, the Hounsfield unit calibration and correction for different CT machine setups is discussed.

2.1. Micro-CT and animal scanning

2.1.1. CT and micro-CT

Computed tomography (CT) uses X-rays to generate cross-sectional, two-dimensional images of the body. For clinical CT machines, the images are acquired by rapid rotation of the X-ray tube 360° around the patient. The transmitted radiation is then measured by a ring of sensitive radiation detectors located on the gantry around the patient (Figure 2.1.1). The final image is generated from these measurements utilizing the basic principle that the internal structure of the body can be reconstructed from multiple X-ray projections. The source-detector geometry and the number of detector
elements are fixed and selected which limits the in-plane resolution (Jackson and Thomas, 2004). The resolution, or the size of the smallest feature that can be reliably and accurately viewed, for current clinical CT is roughly 500 µm.

![Figure 2.1.1. Patient in a clinical CT. (Jackson and Thomas, 2004, Chapter 1)](image)

Micro-CT was pioneered by Feldkamp et al (1989) and the resolution of a micro-CT could be on the order of several microns. Besides the resolution, another major difference between clinical CT and industrial micro-CT is in the machine setup. Most micro-CT machines keep both the X-ray source and detector fixed while rotating the specimen. Figure 2.1.2 demonstrates a typical layout of an industrial micro-CT machine in which the specimen rotates 360° to accomplish the scan.
2.1.2. CT theory

Every acquired CT slice is subdivided into a three-dimensional matrix of volume elements (voxels). Each voxel has been traversed during the scan by numerous X-ray photons and the intensity of the transmitted radiation measured by detectors. From these intensity readings, the attenuation value of the tissue at each point in the slice is calculated. According to the CT theory, a denser material absorbs more X-ray energy, thus should result in a darker region in the image. The original image is often inverted to render an image with bright region representing dense material. Thus, the hard tissue is displayed as white while the soft tissue is dark.

Each voxel of a CT scan is assigned a numerical value (CT number), which is the average of all the attenuation values contained within the corresponding voxel. The term ‘grayscale’ is used to denote the CT number. The image grayscale does not represent the actual density information for the scanned specimen since it is subjected to change.
according to the machine setup which controls the image brightness and image contrast. Even for a consistent configuration, a single bone tissue specimen could also result in different grayscale when scanned with and without flesh. This puts the grayscale value into question when the density information is required and the subject is scanned under different conditions (*in vivo, in vitro* etc) as in this research. To acquire consistent information, the Hounsfield unit is introduced to interpret the tissue density. The Hounsfield unit is calculated by comparing the grayscale value to the attenuation value of water and air phantoms using.

$$HU = 1000 \frac{G - G_w}{G_w - G_a}$$  \hspace{1cm} (2.1-1)

where $G_w$ and $G_a$ are the grayscale value of water phantom (0 HU) and air phantom (-1000 HU) (Hounsfield, 1973). Phantoms are reference targets containing volumes of known material placed within the field of view of the scan.

**2.1.3. Scanning equipment and peripheral hardware**

For this study, micro-CT scanning was performed using a custom-made micro-CT scanner (Model ACTIS 200/225 Ffi-HR CT/DR system. BIR inc., Chicago, IL with built-in X-ray system FXE 225.20, Fein Focus U.S.A). The scanner consists of a specimen manipulator (turn table), an X-ray source, an image intensifier and a CCD camera (Figure 2.1.3). In addition, an anesthesia machine (Landmark Model VSA-2100, Hallowell Model 2000) was added to the scanner so that *in vivo* scanning could be performed with the animals sedated to minimized unwanted motion (Figure 2.1.3).
Figure 2.1.3. Micro-CT machine layout with a living rabbit sitting in a bucket on the turn table. The CCD digital camera is located at the right hand side of the image intensifier which is not shown in this figure.

The micro-CT system is able to scan at a resolution of 5 µm. The resolution of an actual scan is dependent on the physical setup (Figure 2.1.4). Since the X-ray source is a type of cone beam, it shoots X-rays cross the scanned object and the passing X-ray reaches the image intensifier. To achieve the finest detail output, the ratio of the source to image intensifier distance (SID) to the source to object distance (SOD) should be maximized. At the same time, one-half of the object diameter (technically it is slightly larger than that) should occur in the scan range which is the area between the two dashed lines in the Figure 2.1.4. Because the distance between the X-ray source and the detector is fixed and the image intensifier size is also fixed, the resolution is then determined by the diameter of the scan target. As shown in Figure 2.1.4, tube 1 (T1) has a higher resolution than tube 2 (T2). The \textit{in vitro} scans (scans of bone cube that was cut from
distal femur condyle) and ex vivo scans (scans of distal femur without flesh) have a resolution of 14 µm. The \textit{in vivo} scans have a resolution of 28 µm.

![Diagram](image)

Figure 2.1.4. The scan resolution is determined by micro-CT layout geometry (not to scale).

2.1.4. \textbf{Animal species used in the research}

The selected living animal must be small enough to be positioned on the specimen manipulator within the scanner to make an \textit{in vivo} scan. This eliminates the possibility of using the medium size animals such as goats or pigs. According to the continuum assumption (Harrigan et al. 1998), which defines the sample size for cancellous bone above which the sample is able to represent the overall material properties continuously, 3 to 5 mm in each direction is the lower limit of the sample size. Thus, the common lab rat could not be used. New Zealand white rabbits were chosen for this study. The condyle of rabbit distal femur provides sufficient trabecular bone for the apparent modulus and strength studies. In Figure 2.1.5, a reconstructed 3-D condyle of a rabbit
distal femur is shown on the left. A trabecular bone cube was cut from a region of interest (ROI) which is shown on the right.

Figure 2.1.5. A rabbit condyle model reconstructed from micro-CT slices (left) with a trabecular cube cut from it (right).

2.2. Image registration

2.2.1. Purpose of image registration

Image registration, also denoted as image alignment, is a fundamental task in the processing of multimodality three-dimensional medical images or of multiple scans of the same specimen taken at different times, as implemented in this study (Hill et al., 1994; Studholme et al., 1996, 1999; West et al., 1997; Woods et al., 1993). When multiple scans are made at different time points, it is difficult to guarantee analysis of the same ROI. To take full advantage of in vivo scans, a reliable image registration technique was developed. The characteristics of bone trabeculae, including small dimension size and geometry variance problems caused by bone growth or adaptation, eliminate the use of most current image registration techniques, such as stereotactic coordinate frames (Fox et
al. 1985); fiducial landmarks (Kremser et al 1997); anatomical landmarks (Banerjee 1995; Hartkens et al. 2002) and structure based techniques (Bernon et al. 2001; Suganoa et al. 2001). An image registration technique based on maximization of mutual information has been proven to be efficient and precise (Collignon et al., 1995; Maes et al., 1997, 1999; Studholme et al., 1999). For this study, a registration program, based on the maximization of mutual information technique, was developed and the registration quality validated.

2.2.2. Mutual Information Theory

Mutual information (MI) is a basic concept from information theory. It has been used to measure the statistical dependence between two random variables or the amount of information that one variable contains about the other. The MI registration criterion states that the mutual information of the image intensity values of corresponding voxel pairs from two similar images (a reference image and a floating image) is maximal if the images are geometrically aligned (Maes et al., 1997). Studholme et al (1999) used terminology of entropy to explain the mutual information definition (Figure 2.2.1).

![Figure 2.2.1. Description of mutual information by entropy (Studholme et al. 1999)](image)

To explain the mutual information applied in image registration, the terminology of image intensity distribution is used rather than entropy. Let $r(s)$ denote the image intensity in the reference image at position $s$. The floating image is transformed by a
parameter \(a\). Let \(f_{Ta}(s)\) denote the image intensity in the floating image at position \(s\) after the transformation. The joint image intensity histogram \(h_a(f,r)\) of the overlapping volume \(V_a\) with the transformation \(a\) of the floating image is computed by binning the image intensity pairs \((r(s), f_{Ta}(s))\) for all \(s \in V_a\) (Figure 2.2.2). For the grayscale value of the voxel at each location \(s\) of reference image, find the grayscale value of the voxel at the corresponding location from the floating image, the two grayscale values were put together as a pair which was defined as the binning procedure.

![Figure 2.2.2. Description of the joint intensity calculation. In the left figure, the floating image was transformed by a parameter \(a\). Right figure explains how to calculate the joint image intensity histogram by binning \(r(s)\) and \(f_{Ta}(s)\). (Right image: Studholme et al. 1999)](image)

The joint image intensity distribution \(P_{FR,a}(f,r)\) is estimated by normalization of \(h_a(f,r)\) using

\[
P_{FR,a}(f, r) = \frac{h_a(f, r)}{\sum_{f, r} h_a(f, r)} \quad (2.2-1)
\]

The marginal image intensity distributions \(P_{F,a}(f)\) and \(P_{R,a}(r)\) are estimated using

\[
P_{F,a}(f) = \sum_r P_{FR,a}(f, r)
\]
\[
P_{F,a}(r) = \sum_f P_{FR,a}(f, r)
\]
The MI registration criterion \( I(a) \) is evaluated by

\[
I(\alpha) = \sum_{f,r} P_{FR,\alpha}(f,r) \log_2 \left( \frac{P_{FR,\alpha}(f,r)}{P_{F,\alpha}(f)P_{R,\alpha}(r)} \right)
\]  

(2.2-3)

where the optimal registration parameter \( a^* \) is defined by

\[
\alpha^* = \arg \max_\alpha I(\alpha)
\]

(2.2-4)

where \( a \) is the vector with which the mutual information reaches its maximal.

### 2.2.3. Implementation

To use the available minimization searching algorithms, the maximization of mutual information was inverted to become a minimization problem. The Powell multi-dimensional minimization algorithm was selected for its efficiency with large size files. In addition, this method is faster in all likely applications than other methods such as the downhill simplex method (Press et al. 2002). This is partly because there is no derivative used when finding the next direction. The six degrees of freedom (DOF) are minimized in the order of \( T_x, T_y \) and \( R_z \) coefficients followed by the \( R_x, R_y \) and \( T_z \) coefficients as recommended by Lin et al. (1996). The subscripts \( x, y, z \) refer to the three axes of a Cartesian coordinate system and \( T \) means translation and \( R \) means rotation. When calculating the joint histogram, as shown on the left figure in Figure 2.2.2, the grayscale of each pixel in the reference image and the grayscale of the pixel at the corresponding location in the floating image must be determined. For example, when registering a 2D image, the grayscale value of a pixel at location \((\alpha, \beta)\) in the reference image and the grayscale value at the location \((\alpha, \beta)\) in the floating image must be determined. However, because the floating image was translated and rotated continuously, it is very possible
that location $(\alpha, \beta)$ drops between two or more voxels. Thus an interpolation method, Nearest Neighbor (NN), was employed to interpolate the grayscale value at location $(\alpha, \beta)$.

Computer memory and running efficiency were found to be the main concerns during implementation. The number of matrices was minimized during programming and sparse matrix was used to minimize the amount of memory used. Matrix manipulation was used whenever possible other than the LOOP command to improve the efficiency. In addition, the program provides users with image degradation options which can degrade the image before actual registration. Even though the input files are mostly 16bit images, users have options to make the program run in three modes: 16bit, 8bit and binary. In 16bit mode, the program works on the original file types, which is not efficient. No significant accuracy improvement was observed with this mode compared to other modes. The 8bit mode proved to be efficient with accepted accuracy. The binary mode, i.e. black and white model, is particular useful when significant noise is present in the image such as a pure black scanning background. By segmenting the image into monochrome, only bone voxels are white and all others are black, thus the noise was eliminated. A typical in vivo micro-CT scan of a rabbit distal femur condyle is shown in Figure 2.2.3. In the left figure, bone, marrow and flesh could be taken as useful information. While the air, plastic container and out of scan range background are all noise because this information may not be present at the corresponding location of the other image to which the current image will be registered. The right image is the corresponding black-white image for which all noise was eliminated and only bone information is left.
Figure 2.2.3. Information and noises present in a typical *in vivo* micro-CT scan (left). The corresponding black-white image is shown in the right figure where all noises are eliminated by converting the image to monochrome.

2.3. Registration validation

2.3.1. Necessity of registration validation

The registration technique developed based on the maximization of mutual information theory belongs to the rigid registration domain for which only spatial error is corrected. The reference and floating 3D images are supposed to be the same for this category of registration technique. The *in vivo* bone scans taken from a living animal at different time points are different in geometry structure because of bone growth or loss. The growth effect, which was defined as a geometry variance problem during registration, refers to the trabecular bone geometry change caused by bone growth or bone turnover. Thus, the images to be registered are not exactly the same. The accuracy of registration is thus in doubt and must be validated.

The two most widely used registration validation methods are the physical (fiducial) landmark method (Benameur et al. 2003; Chui et al. 2003; Collignon et al. 1995b; Jan et al. 2002; Maes et al. 1997; Studholme et al. 1999; Thomas et al. 2003) and the
predetermined manipulation parameters method, which works by comparing the predetermined vectors that are added to the images as errors and the registration rendered vectors. These two sets of vectors should be close in value but with opposite signs for good registration (Cai et al. 1998; Collignon et al. 1995a; Guéziec et al. 2000; Reichenbach et al. 2002). The landmark method is limited by the sample size, the ability to attach landmarks to small samples, and the uncertainty of landmark movement during bone growth. The predetermined manipulation parameter validation method has difficulty mimicking bone changes (such as growth), thus the validation would not be accurate if the registration is supposed to be used to evaluate changes in bone over time. A new registration validation method was developed to validate the registrations of rabbit femur trabecular images. This automated voxel grayscale based technique takes advantage of the similarity of the net bone formation percentage (%NBF) in the adjacent regions within a bone.

2.3.2. Method description

A small region, A1 (Figure 2.3.1), from the reference image is selected and the bone volume fraction \( \frac{BV_{A1}}{TV_A} \) calculated. A region A2, which has the same size and same position as A1, is selected from the registered floating image and the bone volume fraction \( \frac{BV_{A2}}{TV_A} \) calculated. The %NBF or volume fraction ratio \( R_A \) is given by

\[
\%NBF = R_A = \frac{BV_{A2}}{TV_A} / \frac{BV_{A1}}{TV_A} = \frac{BV_{A2}}{BV_{A1}}
\]

(2.3-1)

A region B1 from the reference image, in close proximity to A1, is selected together with B2 in the registered floating image and %NBF calculated using

\[
\%NBF = R_B = \frac{BV_{B2}}{TV_B} / \frac{BV_{B1}}{TV_B} = \frac{BV_{B2}}{BV_{B1}}
\]

(2.3-2)
Figure 2.3.1. Slices chosen from reference image (left) and corresponding registered image (right).

For well registered images, %NBF for regions A and B, i.e. $R_A$ and $R_B$, should be approximately equal. For convenience, $R_A / R_B$ is defined as the Ratio of Percentage (RP). A good image registration will have a mean RP of approximately 1.0 with a small standard deviation. If there is no geometry variance involved in the registration, such as for in vitro scans, it is unnecessary to calculate RP. Calculating %NBF is sufficient. If the %NBF is close to 1.0 everywhere, then a good registration is achieved.

Two parameters, the selected region size (SRS) and selected region distance (SRD, distance between A1 and B1 or A2 and B2 shown in Figure 2.3.1) must be optimized for the validation method to work properly. The optimal size for the selected region was determined using three rabbit distal femur CT scans with 28 µm nominal resolution. Unregistered images were created by adding random translation and rotation errors to copies of the three images. The original images and the artificially un-registered images were then registered. The SRS was varied from 5 to 50 voxels in all three dimensions. The RP standard deviations for all three experiments decreased as the SRS increased as shown in
Figure 2.3.2. An SRS between 5 and 20 voxels is defined as Error-Sensitive-Zone where RP standard deviations are high because of errors produced by the registration process, the re-slicing procedure and the image quality. The SRS between 35 to 50 voxels is defined as the Error-Dull-Zone where the validation method is less sensitive to registration error. Figure 2.3.3 shows the RP standard deviation for both the registered and un-registered images with a significant improvement for the registered images.

In the second experiment, one image was selected and its exact copy was translated in x, y and z directions by -2, -1, 1, and 2 voxels thereby creating 12 shifted images (3 directions × 4 offset=12). Each of these translated images together with the original image were used as a ‘registered’ pair and evaluated. The SRS was varied from 5 to 50 voxels in all three dimensions. The purpose of this experiment was to find an SRS with which the validation method could detect a one-voxel registration error. As shown in Figure 2.3.4 - Figure 2.3.6, RP standard deviations in Error-Dull-Zone are similar for images with one or two voxel error shifts. For the validation method, an SRS of 25 was used.
An experiment to determine the effect of the SRD on validation results is similar to the first experiment except that the SRS was fixed at 25 voxels and the SRD varied from 1 to 10 voxels. The validation results are shown in Figure 2.3.7. The linear regression slopes for each experiment are 0.00107, -0.07116, -0.02256 respectively. Since SRD has no significant effect on registration, an SRD of 5 was used for validation. For each validation, twenty selected region groups were used to stabilize RP and the standard deviation. Each group consists of four selected regions A1, A2, B1 and B2 as shown in Figure 2.3.1.

Figure 2.3.4. The validation results for images shifted in x direction.

Figure 2.3.5. The validation results for images shifted in y direction.

Figure 2.3.6. The validation results for images shifted in z direction.

Figure 2.3.7. The SR distance effect.
An experiment was carried out to test the method by simulating in-vivo scans at different points in time. Five artificially un-registered images were created by adding random translation and rotation to the rabbit distal femur trabecular image. During image creation, lower threshold values were used to simulate bone growth. As the added spatial errors were known a priori, the registration error was easily quantified and displayed in Table 2.3.1. Small translation and rotation errors indicate good registration. This result was also expected from the voxel grayscale based method. As shown in Figure 2.3.8, the RP means were approximately 1.0 with standard deviations less than 0.1. The registration was considered accurate and accepted when the RP standard deviation was less than 0.1.

Table 2.3.1 Registration errors for five tests.

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Translation (unit: voxel)</th>
<th>Rotation (unit: degree)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>Y</td>
</tr>
<tr>
<td>1</td>
<td>0.450</td>
<td>0.159</td>
</tr>
<tr>
<td>2</td>
<td>0.365</td>
<td>-0.257</td>
</tr>
<tr>
<td>3</td>
<td>-0.251</td>
<td>-0.215</td>
</tr>
<tr>
<td>4</td>
<td>-0.135</td>
<td>-0.020</td>
</tr>
<tr>
<td>5</td>
<td>-0.166</td>
<td>0.072</td>
</tr>
</tbody>
</table>
The registration translation error was also quantified using the validation method. A registered floating image from the previous experiment was shifted in x, y and z directions by -2, -1, 0, 1, and 2 voxels, thereby creating 12 shifted less-registered images. The RP standard deviations for each shifted image are shown in Figure 2.3.9. The smallest standard deviation occurred for the non-shifted registered image. When the registered image was shifted in any direction, the RP standard deviation changed slightly indicating that the global translation error of the registration is less than one voxel. Rotation error was not evaluated explicitly. Rotation can be taken as a particular kind of shifting which is not along the spatial axis but along the peripheral direction.

![Figure 2.3.9. The RP standard deviation for manipulated images.](image)

This voxel based registration validation method can be used to validate micro level image registration where existing registration validation methods do not apply. The results showed that the registration method based on maximization of mutual information algorithm worked well in registering high-resolution trabecular bone images with
geometry variance problem. The global error of the registration was shown to be less than one voxel along spatial axes.

### 2.4. Image preprocessing

#### 2.4.1. Image degradation

In finite element analysis (FEA) of trabecular bone, because of the complexity and irregularity of the trabecular network, the finite element models (FEM) are commonly constructed by converting three-dimensional micro-CT or other scans directly into finite element models. A voxel conversion technique was implemented to accomplish this procedure, in which each element in the finite element model was converted from the corresponding voxel in the image stack, which will be elaborated in the next chapter. For a 4 mm trabecular cube scanned with 14 µm resolution and the finite element model would have 11.7 million elements assuming 50% volume fraction, which is reasonable for a rabbit distal femur. For a nonlinear analysis, such a model could hardly be solved within a reasonable time frame. Image degradation increases the image voxel size, thus increasing the corresponding element size in the model and reducing total number of elements. The apparent properties of trabecular bone can be determined with sufficient accuracy when the element size is less than half of the trabecular thickness (Harrigan et al., 1988; Homminga et al., 2001; Muller et al., 1996). Since the trabecular thickness for the rabbit distal femurs is approximately 170 µm, an element size of 56 µm, which is one-third of the trabecular thickness, is small enough to achieve an accurate result (Harrigan et al., 1998) and the model size is also small enough to run efficiently.
The performance of two degradation methods were evaluated with respect to both homogeneous and heterogeneous finite element models. The region average (RA) image degradation method is widely used (Bayraktar et al., 2004; Bourne et al., 2004; Niebur et al., 1999). The degraded voxel is generated by combining a particular number of adjacent voxels of the original models and averaging their grayscale values. (Figure 2.4.1a). A voxel expansion (VE) image degradation method is proposed for which the degraded voxel is generated by directly expanding a single original voxel. The skipped voxels are discarded (Figure 2.4.1b).

Three New Zealand white rabbit distal femurs were scanned in vitro with 14 µm nominal resolution. Six 1.75 mm cubes were generated from the three image stacks. The continuum requirement (Harrigan et al., 1998) for trabecular bone analysis does not apply because all the result comparisons were made between the models which were degraded.
from the same model by different degradation methods. The voxel size of each model was degraded five times by both degradation methods to 84 $\mu$m. The degradation performance was evaluated primarily based on the apparent stiffness from finite element analysis as well as the trabecular bone indices (BV/TV, Tb.Th) and grayscale distribution. The Otsu method (Otsu, N., 1979) was used to segment the image stacks for the trabecular indices calculation. The bone indices and grayscale distribution evaluation are helpful to examine the underlying effect of the degradation methods on the finite element analysis.

The percentage errors for volume fraction and Tb.Th, which were calculated using image analysis software (Volume Graphics), showed significantly difference between the methods (Figure 2.4.2). The VE method generated significantly less error than the RA method, especially for the bone volume fraction comparison, even though the errors of both tend to increase as the element size increases.

![Figure 2.4.2. Trabecular indices comparison between two degradation methods.](image)

The two methods have different effects on the grayscale distribution. Unlike the VE method which does not change the histogram significantly (Figure 2.4.3 B, D), the RA method shifts both sides of the histogram to the center (Figure 2.4.3 A) where the black
dots drop within the red line which represents the original histogram. The shifting at the right side softens the heterogeneous finite element models in which the material properties are directly or indirectly related to the voxel grayscale values. Because the left side of the histogram represents the marrow, it is not involved in the bone model. Thus, any effect of the left side shifting is not as important.

Figure 2.4.3. Grayscale distribution comparison between two methods. A: Grayscale distribution of the RA degraded model and the original model. Corresponding errors are displayed in C. The same content for the VE method is displayed in B and D.
For finite element analysis, both homogeneous models \((E=10 \text{ GPa})\) and heterogeneous models \((E=20 \times (\text{tissue grayscale})^{1.5} \text{ GPa})\) were tested. The models were reconstructed from the micro-CT scans and meshed with 8-node hexahedron element (C3D8). The axial boundary condition was set as fixed at one end and a 0.2% axial strain loaded at the other end. The apparent stiffness of the degraded models was compared to that of the original models (Figure. 2.4.4). There was no obvious FEA error trend for the VE method. For the RA method, the homogeneous models were stiffened by the increased volume fraction; the heterogeneous models were softened because the grayscale soften effect discussed above was overwhelming.

Even though the VE method works by discarding information, it is better than the RA method which works by keeping all the information in the form of averaged grayscale. The bone tissue moduli of the heterogeneous models were calculated from Hounsfield unit which is grayscale related. Thus, the VE method for model degradation was chosen to avoid the grayscale softening effect.

Figure. 2.4.4. Apparent stiffness error between two degradation methods. Homogeneous models (A). Heterogeneous models (B).
2.4.2. Image segmentation

One of the problems with using micro-CT images to construct trabecular bone models is that the image must be segmented first to distinguish bone tissue from marrow. The best method to determine this threshold is by Archimedes principle (Ding et al., 1999), which can accurately determine the bone volume by comparing the sample weight in air and in water. However, applying this method is relatively time-consuming and not practical for in vivo segmentation. A typical histogram of a trabecular bone is demonstrated in Figure 2.4.5. In the histogram, the left peak represents the marrow and the right peak represents the bone tissue. The accuracy of the segmentation depends on how good the threshold is determined such that the resulted trabecular structure is neither underestimated nor overestimated.

![Figure 2.4.5. A typical grayscale histogram of a trabecular bone cube.](image)

Generally two categories of threshold methods, global threshold and local threshold, have been reported in the literature. Global threshold is the most widely used...
segmentation technique. A single micro-CT number is chosen, above which all voxels are marked as bone and all remaining voxels are marked as non-bone. The segmentation procedure for the global threshold is relative simple and fast. The other category denoted as local threshold technique is sought to improve the segmentation quality when threshold accuracy is not satisfied by the global method because the global threshold was reported to result in loss of thin trabeculae and over-sizing of thick trabeculae (Waarsing et al., 2004). There have been several local threshold techniques developed to optimize segmentation quality (Dufresne, 1998; Elmoutouakkil et al., 2002; Kuhn et al., 1990; Waarsing et al., 2004). Despite the advantages of the local threshold technique, Waarsing et al. (2004) claimed that at high resolution, conventional global segmentation methods gave near exact representations of the bone structure. In this research, the segmentations were all done at the micro-CT scan’s original resolutions (14 µm for in vivo scans and 28 µm for in vitro scans) which are high enough for the global threshold to be accurate.

The Otsu algorithm (Otsu, 1979) was used in this study; this has been proven to be valid for the micro-CT images (McCreadie et al., 2001; Muller et al., 2002; Uchiyama et al., 1997) was used. The Otsu method is a nonparametric and automated method which works by maximizing the separability of the resultant classes in gray levels. When it is applied to trabecular bone image, it takes the marrow as the background and the bone tissue as the object or vice versa to separate from each other. Thus any other information which is not marrow or trabecular tissue is considered noise and should be avoided.

Matlab has a built-in function for the Otsu method to determine image threshold value. The function converts the image to 8bit first, but the threshold value determined from the 8bit image was significantly lower than the one determined from the original
16-bit image, which was proven to be accurate. Thus the built-in function was modified to use the original 16-bit images to determine the threshold value.

### 2.4.3. Unconnected voxel elimination

After segmentation, the micro-CT scans always have some voxels geometrically disconnected from the main volume. These unconnected voxels must be eliminated, or numerical problems for the following finite element analysis such as unconstrained rigid body modes may result.

In three dimensions, a voxel could be taken as geometrically connected when any of the following requirements is satisfied (Figure 2.4.6).

1. At least one face connects to the main model.
2. At least two edges connect to the main model.
3. At least one edge and one node connect to the main model.
4. At least three distinct nodes connect to the main model.

![Figure 2.4.6. Description of the voxel connection. Blue represents main structure.](image)

These four rules are summarized into one rule that whenever a voxel has at least 3 corner nodes connected to the main structure, the voxel could be taken as geometrically connected. But practically only the first connection rule was considered. For finite element analysis, the node or edge connection should always be avoided to prevent severe stress concentration and mesh distortion problem. Practically, it is found that node
and edge connection elements are always few or even absent in the model. Thus considering only the face connection element doesn’t result in a significant loss of connected elements in the model.

A union-find algorithm (Sedgewick, 1998) was used to group the connected voxels into different unions, as shown in Figure 2.4.7, based on the defined connection criterion. Each union was assigned a label. As for the trabecular bone stack, the main structure of the bone tissue is always the largest one among the unions. In Figure 2.4.7 the largest one ‘union A’ is taken as the main structure and the rest union B and C will be eliminated as unconnected voxels. The whole procedure is fully automatic and thus can be used in a batch process.

Figure 2.4.7. Description of the union-find algorithm. A was kept as main structure and B, C were eliminated as unconnected elements

Normally the unconnected voxels only account for a very small portion of the whole trabecular bone voxels. On the examination of 12 trabecular cubes (4.5 mm dimension size in average), which are for later trabecular tissue material properties
calibration, the amount of elements ranged from 126,372 to 329,374, and corresponding unconnected element from 9 to 374. The unconnected ratio was 0.0608% (Std. Dev. 0.0628%). With this small ratio, the potential effect of discarding unconnected elements on the trabecular bone mechanical properties could be safely ignored.

2.5. Hounsfield converting

2.5.1. Hounsfield calculation

In a CT image, each pixel is assigned a numerical value (grayscale value), which is the average of all the attenuation values contained within the corresponding voxel of the scanned specimen. This number is compared to the attenuation value of water and air and displayed on a scale of arbitrary units named Hounsfield units (HU) (Jackson and Thoms 2004). The Hounsfield unit rather than the grayscale was used in this research because of its reliability and stability in representing the bone density information. To convert the grayscale to Hounsfield unit by Eq. 2.1-1, the corresponding water and air grayscale in the same scan were required. Two phantoms, water and hydroxyapatite (HA) were scanned with all bone samples. A typical scan with both phantoms is shown in Figure 2.5.1. During an actual calculation, the air grayscale was calculated from a region that was as far as possible from any objects, such as the container’s wall or bone sample, to avoid any possible streak (beam hardening) artifact. The hydroxyapatite was not used in the Hounsfield calculation.
2.5.1. A typical bone cube scan with water and hydroxyapatite (HA) phantoms. HA is not used in the Hounsfield calculation.

2.5.2. Hounsfield correction for inconsistent CT machine setup

The accuracy of the Hounsfield unit is critical to the current research. The material grayscale in a CT image depends on material thickness, material density, atom number and photon energy. The last two are affected by the setup of voltage and amperage which are normally adjusted for good quality of images. Even though a consistent CT machine configuration was required in this study, occasionally it could not be achieved because some of the samples were borrowed from other studies which used different setup of the CT machine. Cozzi et al. (1998) observed in their study that a voltage variation from 100 to 140kV could result in a significant difference (~300HU) at high densities ($\rho=1.827\text{g/cm}^3$). Currently, there is no literature that describes how amperage and voltage affect Hounsfield unit in detail with respect to material density and voltage or amperage level. There is no method given in the literature on how to convert Hounsfield units from one configuration of voltage and amperage to another. Thus it was necessary to develop a method to accomplish this kind of conversion between configurations.
A cortical bone specimen cut from bovine femoral head ($\rho=1.822\,\text{g/cm}^3$) and three fiberglass composite cubes (30 pcf, 40 pcf, 50 pcf) were scanned together at 14 $\mu\text{m}$ resolution. A water phantom and a hydroxyapatite phantom were included in the scan. Four scans were made under the same voltage of 82kV with amperages of 51, 73, 97, 107 mA. Another four scans were taken under the same current of 54mA with voltages of 70, 82, 110, 125 kV. The voltage and amperage were chosen randomly within the workable range of the machine. Each scan contains 17 slices from a single rotation of the micro-CT machine’s turntable. The eight scans were taken continuously with different voltage and amperage setup. There was no movement of any physical part between scans, which guaranteed the eight scans containing exactly the same regions of the specimens. The outlined rectangular regions in Figure 2.5.2 indicate the different material scanned.

![Figure 2.5.2. Specimens and phantoms layout. The squares indicate the regions for calculating grayscales for particular items.](image)
As shown in Figure 2.5.3a, the grayscale tended to increase for all materials as the voltage decreased. For high density material, hydroxyapatite and cortical bone, the curves became more and more nonlinear as the voltage decreased. The amperage affected the grayscale in a different way as shown in Figure 2.5.3b. As the amperage increased, the grayscale level decreased linearly within a limited range and the curves are roughly parallel to each other for all materials.

![Figure 2.5.3. a: Voltage effect on grayscale values. b: Current effect on grayscale values. The curves for Sawbones are between the water and air curves.](image-url)

The grayscale (G) was converted to Hounsfield unit by Eq 2.1-1. In Figure 2.5.3a, the curves of low density Sawbones are parallel to that of air and water. Thus, in Figure 2.5.4a where the voltage effect on Hounsfield unit is displayed, the curves of Hounsfield units of Sawbones are nearly flat. The curves are actually decreasing with slight slopes as the voltage increases (mean error 62.4 HU between 70 and 125kV). On the other hand, the curves of hydroxyapatite and cortical bone inherit the nonlinear feature of high density material curves in Figure 2.5.3a, which resulted in significant Hounsfield unit error (error >1000HU). For the amperage effect (Figure 2.5.4 b), the curves are all nearly...
horizontal despite densities (mean error 30.6 HU between 51 and 107mA), which was resulted from the parallel nature of the curves in Figure 2.5.3 b.

As demonstrated, the errors of Hounsfield unit were significant between different voltage configurations for high density materials but did not vary with current. The hydroxyapatite (HA) and cortical bone curves (Figure 2.5.4a) were represented by power relations given by ($R^2=0.99$ and 1 respectively)

$$H_{HA} = 54651V^{-0.5562}$$  \hspace{1cm} (2.5-1)

$$H_{cor} = 64188V^{-0.7228}$$  \hspace{1cm} (2.5-2)

Figure 2.5.4. a: Voltage effects on Hounsfield unit. b: Amperage effect on Hounsfield unit. The lower three flat curves for sawbones with different densities.

As demonstrated, the errors of Hounsfield unit were significant between different voltage configurations for high density materials but did not vary with current. The hydroxyapatite (HA) and cortical bone curves (Figure 2.5.4a) were represented by power relations given by ($R^2=0.99$ and 1 respectively)

$$H_{HA} = 54651V^{-0.5562}$$  \hspace{1cm} (2.5-3)

$$H_{cor} = 64188V^{-0.7228}$$  \hspace{1cm} (2.5-4)
The amperage effect on Hounsfield unit, as shown in Figure 2.5.4b could be ignored, because the amperage effect was relatively insignificant and the residual errors after corrections shown in Figure 2.5.5, were small.

It was reasonable to assume the Hounsfield unit of materials similar to bone would all follow power relations with respect to voltage in the form of \( H = aV^b \) and would not depend on current. Supposing that a material \( m \) was scanned with voltage \( V_m \) and resulted in \( H_m \) HU, the Hounsfield unit of hydroxyapatite (\( H_{HA} \)) and cortical bone (\( H_{cor} \)) at voltage \( V_m \) are formed by substituting \( V_m \) into Eqs 2.5-1 and 2.5-2 and solving for the coefficients \( a \) and \( b \). The power coefficient ‘b’ for material \( m \) is found using

\[
\frac{b - (-0.7228)}{-0.7228 - (-0.5562)} = \frac{H_m - H_{cor}}{H_{cor} - H_{HA}} \tag{2.5-5}
\]

while coefficient ‘a’ is calculated using

\[
a = H_m / V_m^b \tag{2.5-6}
\]

The Hounsfield unit for this material at voltage \( V_n \) is calculated using

\[
H_n = aV_n^b \tag{2.5-5}
\]

To validate this correction method, another scan was made using three configurations: 1) 70kV 114mA; 2) 82kV 97mA; and 3) 110kV 73mA. Two cortical bone specimens, one from the bovine femoral head and another from the rabbit distal femur shaft, were included in the scan. Three testing corrections were made (Figure 2.5.5). Each of the three corrections took one micro-CT configuration as the original setup and converted it into the other two configurations. The converted Hounsfield units were compared to the actual Hounsfield units. The mean absolute errors for the three
corrections are 34.54, 55.51, 30.26 HU respectively and corresponding percentage errors are 1.1%, 1.6%, 1.0%.

Figure 2.5.5. Predict and true Hounsfield unit for the validation scan.
CHAPTER 3. MATERIALS AND METHODS

Mechanical experiments were used to measure the trabecular bone tissue properties and validate the accuracy of the trabecular bone finite element models. This chapter begins by introducing how the trabecular bone samples were prepared for mechanical testing and how the mechanical experiments were carried out. Next, finite element model construction, finite element types, material characteristics, nonlinearity consideration and boundary conditions are discussed. The material stiffness and strength heterogeneity calibration are then illustrated in detail. Finally, the last and most important section of this chapter demonstrates the experimental design for evaluating the trabecular bone osteoporosis by nonlinear heterogeneous finite element models.

3.1. Bone cube cutting and mechanical experiment

3.1.1. Trabecular cube excision

Trabecular cubes were cut from the lateral and medial condyles of the rabbit distal femur. The condyles carry force mainly in anterior-posterior and superior-inferior direction (E1 and E2 in Figure 3.1.1). Bourne et al. (2002) showed that there was no significant mechanical properties difference between E1 and E2 directions. For the actual cutting, a trabecular cube of 4 mm was excised from both lateral and medical condyles. The anterior-lateral plane of the cube was chosen normal to the femur shaft, which was for the convenience of the later registration. Also the cube location was chosen as close
as possible to the posterior region of the condyle, which was relatively denser than the anterior region. The anterior-posterior direction of the samples was kept roughly along the trabecular directions as shown in Figure 3.1.2.

A low speed diamond saw (BUEHLER, Lake Bluff, IL) was used to cut the bone cubes (Figure 3.1.3). The saw was operated at a very low speed (approximately 150 rpm) and the blade was kept hydrated during cutting to minimize bone damage due to drying or heating. The distal portion of the rabbit femur is shown in Figure 3.1.4. The first cut was made perpendicular to the femur shaft. The second cut split the lateral and medial portion of the condyle. The third cut separated two small portions of the condyle from the rest of the femur (Figure 3.1.5). The lateral and medial condyles were then glued to two stainless steel plates. The following four cuts were demonstrated in Figure 3.1.6. The last cut was made parallel to the first cut and separated the sample from the stainless steel plate (Figure 3.1.7). The cube surfaces were marked with different colors as a reminder of the anatomic directions (Figure 3.1.8).
Figure 3.1.3. Low speed diamond saw

Figure 3.1.4. A rabbit distal femur.

Figure 3.1.5. First three cuts in excising bone cube.

Figure 3.1.6. The separated lateral and medial condyles were glued on steel plates. The 4th to 7th cut were shown with blue lines.

Figure 3.1.7 The plate with the sample was attached on the saw; the last cut was parallel to the first one.

Figure 3.1.8. The excised trabecular cube with the green color represents the distal direction and purple for the anterior.
3.1.2. Mechanical experiment

Two types of mechanical experiments, platen test and end-cap test, are commonly employed for testing trabecular bone mechanical properties. The end-cap technique consists of gripping (usually glued in place) a uniform diameter cylindrical specimen in brass end-caps and calculating modulus based on the deformations measured using an extensometer attached to the end-caps (Figure 3.1.9). This type of experiment was reported to be accurate and particularly suitable for relative large specimens (Jacobs et al., 1999; Keaveny et al., 1993, 1994; Odgaard and Linde, 1991). The platen compression test (Figure 3.1.10), in which a cubic or cylindrical specimen is compressed between two parallel smooth platens, has been used extensively for previous studies (Bourne et al., 2004; Hou et al., 1998; Yeni et al., 2001). The platen test is relative easy to implement and particularly suitable for small specimens as used in this research. The interfaces between the platen and bone sample were lubricated to minimize friction. In addition, when both mechanical tests and finite element analyses are applied to analyze the same specimens, the consistency of the boundary conditions is important for the FEA to accurately represent the mechanical test. Overall, the platen test is more straightforward to be simulated in finite element analysis than the end-cap test. Thus the platen compression test was chosen for this research.
Two platens made of 304 stainless steel were mounted on the material testing system (MTS) machine (Figure 3.1.11). The platen surface was polished giving a smooth and flat surface for compression testing. The platen was lubricated with mineral oil to minimize any frictional end effects. A 25.4 mm gauge-length extensometer was attached to the platens to record the displacement (which can be related to the strain) during test. The platens are much stiffer than bone and their deformation is neglected as all deformation is specimen deformation. Compared to the load frame actuator, which is also able to record displacement, the extensometer could avoid most errors caused by the compliance of the platens and the load cell. This is because the extensometer was attached to the platen very close to the sample. During testing, the extensometer was taken as the control channel; thus it is the extensometer recorded strain that controls the movement of the MTS machine.

After the bone cube was carefully positioned in the center region of the lower platen, the actuator was adjusted by hand control to slowly move the upper platen down. Contact between the upper platen and the bone sample was guaranteed by applying a preload of 5-10N. Twenty preconditioning cycles of 0%-0.4%-0% strain were followed by a final
ramp at 500 microstrain/s (measured at gauge length, 0.003 strain/s measured at specimens) that loaded the specimen to 5% strain which guaranteed that the yield point was exceeded. When tested under such a low strain rate, the experiment was effectively quasi-static and the strain-rate effect on strength was not considered (Linde et al., 1991).

A load-displacement curve was obtained from the load cell and extensometer output to determine the trabecular stiffness and strength (Figure 3.1.12). Generally, there are three regions along the curve. The toe region located at the leftmost of the curve is believed to be the end-artifact of the platen test which is mostly caused by imperfect parallel of the upper and lower surface of the bone cube. Bone stiffness was calculated from the slope of the linear region of the stress/strain curve. Normally, the calculation was performed 3 times independently and the averaged number was taken as the final apparent stiffness of the sample. Yield strength and strain were determined by the 0.2% offset method (Beer and Johnston, 1992).
3.2. Pre-processing of the finite element models

3.2.1. Voxel-Mesh conversion (voxel meshing)

Generally there are two ways to generate finite element models from micro-CT scans: the geometry based modeling method and voxel based modeling method. All geometry based methods bear a common feature that the micro-CT scans must be pre-processed to extract the geometry of the bone. This model was then meshed using custom designed or mostly commercial software. Severe drawbacks such as gaps, geometry non-conforming exist at both the surface extracting and meshing steps which prevent a good conversion quality.

Keyak et al. (1990, 1992, and 1993) proposed a new method called voxel meshing to directly generate a mesh from a dataset of stacked images, thus avoiding the geometry extraction step. The two most common applications are the voxel conversion technique providing meshes with brick elements (hexahedron), and the marching cube algorithm.
providing meshes with tetrahedral elements (Camacho et al., 1997). The marching cube
mesh method can mesh the entities with complex geometry. But it leads to a very large
number of rendered elements and nodes compared to hexahedron method.

The voxel-based hexahedron meshing has no geometrical limitations. This
technique usually implies that the element faces are parallel to the three orthogonal axes
defined by the coordinate system of the CT scanner. The resulting mesh is achieved by
arranging the nodes in the form of a cubic lattice and converting one voxel or several
adjacent voxels directly into an 8-node or 20-node brick element (Lengsfeld et al., 1998).
It must be noted that jagged inner and outer surface may cause numerical or convergence
problems (Marks and Gardner, 1993; Rietbergen et al., 1995). However, Keyak et al.
(1993) and Jacobs et al. (1993) have demonstrated that the direct voxel meshing is
satisfactory in predicting the overall behavior of the bone. Guldberg and Hollister (1994)
acknowledged that digital meshes produced increased error at the surfaces. However,
they suggested that the error was acceptable relative to other inaccuracies, such as
material property uncertainty. Because the surface result is not a concern in this study, the
voxel-based hexahedron meshing was considered valid and used in the research.

One technique to accomplish this voxel meshing is to build a cube in a commercial
finite element software, such as ABAQUS, with dimensions large enough to enclose the
object model. The cube is then fully meshed with the desired element size. This is then
followed by eliminating any elements in the model which are not represented by bone at
the corresponding locations in the image stack. Significant user interaction is required
when building the cube, meshing it and finally editing the input file; this is the major
limitation of this method. In addition, the software restrictions sometimes prevent this
method from being implemented. For example, ABAQUS only allows constructing a cube with less than 1 million elements for its academic version.

A programmed direct converting technique was used to generate the trabecular bone finite element models. When a stack of trabecular scans are reconstructed into a 3D solid model (Figure 3.2.1a), it actually renders all the information necessary to construct the corresponding finite element model (Figure 3.2.1b). This direct converting technique uses the matrix index information of the image stack to generate the node and element information for the FE model. As shown in Figure 3.2.2, a voxel is numbered as NOA in a 3D matrix which can also be considered as a spatial coordinate system. N, O, and A represent its location in each dimension of the image stack matrix respectively. The coordinate indices of the eight nodes of the corresponding finite element are calculated from the matrix indices N, O, and A. When generating the input file, the node number and element number were all generated by a Matlab program following the rules of ABAQUS. The element and node definition files and necessary element set and node set files were generated separately from the main input file, which provided a concise main input file for reading or editing. More important, during this voxel-meshing procedure, the matlab program also recorded the voxel grayscale information, which was used to calculate the mechanical properties for individual elements in the later procedures.
Figure 3.2.1. micro-CT scans and degraded images were reconstructed into 3D models (a) and then meshed with hexahedron element in ABAQUS (b).

Figure 3.2.2. The node numbering scheme for the voxel-meshing technique. The voxel index is NOA in the 3D image matrix. All nodes coordinate values were calculated from this index.

### 3.2.2. Element Type of the trabecular model

The selection of element type primarily depends on the element performance and efficiency. Higher order elements do not significantly improve the results of trabecular bone models (Niebur et al., 1999a). Also considering the computing cost, none of the
second order elements were considered. The constant strain tetrahedral element was not considered because of its slow convergence with mesh refinement. It provides accurate results only in general cases with very fine meshing (ABAQUS Technology briefs TB-03-HTB-1). In addition, it will result in much more elements than the hexahedron meshing, which will significantly slow down the solving procedure as mentioned before.

The 8-node brick is the most commonly used element in the literature to mesh trabecular bone model. This element bears a problem called shear locking which makes the element over stiff in bending. It has been demonstrated that there is more bending in lower density specimens than higher density ones (Bayraktar and Keaveny, 2004b). The volume fraction of rabbit distal femur is in the range of 35% to 50% which is higher than other species and anatomic sites. In addition, by aligning loading direction with the trabeculae principal direction, the bending deformation can be significantly decreased (Niebur et al., 1999a). Thus inaccuracy caused by bending can be minimized. In addition, the discretization of the bone architecture, inherent in the tomography process, leads to an underestimate in the calculated elastic modulus (Ladd et al., 1998b). The model will become even softer when it was degraded to larger element size (Niebur et al., 1999a) which was also observed in this study. Ladd et al. (1998b) has concluded that the softening error can be significantly canceled by the excess bending resistance when the eight-node brick element is used in the model.

It must be noted that even though they serve the same purpose, image degradation is different from the mesh coarsening procedure of FEA. The mesh coarsening procedure does not change the model’s geometry detail. The coarse models are normally stiffer than the corresponding fine model. For the image degradation, the geometry of the original
image (model) could not be fully retained (Figure 3.2.3); this is also known as the partial volume problem. As opposed to stiffening the model, Niebur et al. (1999a) has noted in their work that this geometry changing decreases the structural stiffness.

![Image 3.2.3](image.png)

**Figure 3.2.3.** Description of the image degradation, which is different from the mesh coarsening procedure of finite element analysis.

For ABAQUS, there are three commonly used 8-node brick elements. They are full integration (C3D8), linear reduced-integration element (C3D8R) and the incompatible mode element (C3D8I). The C3D8R only has one integration point. It is efficient and very tolerant of distortion, but it tends to be too flexible. In addition, this element type was found to be too sensitive to the element size which could result in serious convergence problem. The C3D8I is an attempt to overcome the problems of shear locking in fully integrated, first-order elements. It can produce results in bending problems that are comparable to quadratic elements but at significantly lower computational cost. A case study was performed to compare the performance of these three types of elements and the results are shown in Table 3.2.1. Assuming the result of the model using element C3D8I is accurate, then the C3D8 achieved a result comparable to the C3D8I but with a significant short time that is comparable to the C3D8R. Thus the full integration element C3D8 was used throughout the research.
Table 3.2.1 Analysis time and result comparison between three element types.

<table>
<thead>
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<th>C3D8</th>
<th>C3D8R</th>
<th>C3D8I</th>
</tr>
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<td>2280</td>
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<td>1.6089</td>
<td>1.6762</td>
</tr>
</tbody>
</table>

3.2.3. Material characteristics

The material properties of the trabecular bone tissue in the finite element models were assumed to be

- Isotropic
- Heterogeneous
- Imperfect yielding
- Asymmetric bone tissue failure strains

For trabecular bone modeling, it is often implicitly assumed that bone tissue anisotropy has a negligible effect on the apparent elastic properties (Cowin, 1997; Niebur et al., 2001; Yeni et al., 2001). Kabel et al. (1999) have further concluded from their work that the anisotropic bone tissue properties have negligible, if any, impact on apparent elastic properties.

The bone remodeling process is more active on the bone surface than in the middle of the trabecular substance. Because bone mineralization is a function of time, normal remodeling results in higher mineral content in the interstitial bone compared to the surface layer. Therefore, trabecular tissue is more accurately defined as a heterogeneous material than a homogeneous one. In previous research, homogeneous material properties were commonly employed to simplify the trabecular bone modeling procedure and good results were reported for this simplification (Hou et al., 1998; Kabel et al., 1999; Keaveny et al., 2001; Ladd et al., 1998a). Some others reported that intra-specimen variations in
bone tissue modulus, if large, might have appreciable effects on trabecular apparent modulus (Jaasma et al. 2002; van der Linden et al. 2001). Bourne et al. (2004) demonstrated that heterogeneous models having tissue moduli unique to each model more accurately predict the trabecular bone elastic behavior than homogenous models. In addition, the final purpose of this research is to noninvasively evaluate the changes of trabecular bone mechanical properties at different times. The variation in apparent mechanical properties can result from either the variation of trabecular bone structure caused by bone growth and turnover or the variation of trabecular tissue mineralization. The heterogeneous model is more suitable than the homogeneous model because it can accurately capture the variations in both of the tissue properties and structures. 

The post-yield hardening of the material is demonstrated in Figure 3.2.4. In both tension and compression, a hardening slope equal to 5% of the elastic modulus was used as proposed by Bayraktar et al. (2004a).

For the trabecular bone tissue level strength, a number of previous studies used the von Mises criterion to model bone tissue yielding (Fyhrie and Hou, 1995; Rietbergen et al., 1998; Silva and Gibson, 1997; Stolken and Kinney, 2003). This criterion assumes
equal tensile and compressive yield properties similar to that for ductile metals (Beer and Johnston 1992). This assumption has been questioned since the 1970’s because cortical tissue is stronger in compression than tension (Reilly and Burstein, 1975), and similar behavior is likely for trabecular tissue (Keaveny, 1997; Morgan et al., 1999; Niebur et al., 1999b). Niebur et al. (2000, 2001) demonstrated that a single symmetric bone tissue yield criterion cannot accurately capture the behavior for apparent tensile, compressive and shear loading. However, once an effective bone tissue modulus is calibrated and uniform but asymmetric tissue failure strains are used, the resulting models can capture the apparent strength behavior to an outstanding level of accuracy. In this study, an asymmetric tissue failure strain criterion was used for accuracy consideration. von Mises strain and principal strain are used for the compressive and tensile yielding determination respectively. For the tensile-compressive strength ratio, Niebur et al. (2000) used 0.6 for bovine bone while Bayraktar et al. (2004a) found 0.63 for human trabecular bone. In this study, 0.50 was used for the tension to compression yield ratio (Bayraktar et al. 2004b, ABAQUS Technology briefs TB-03-HTB-1).

\[
\varepsilon^c_y = 2\varepsilon^t_y \quad (3.2-1)
\]

where \(\varepsilon^c_y\) is compressive yield strain and \(\varepsilon^t_y\) is tensile yield strain.

The effects of variations in Poisson’s ratio are negligible for bone tissue (Ladd et al., 1998b). The elastic Poisson’s ratio was set as 0.3 and plastic Poisson’s ratio 0.47 (ABAQUS Technology briefs TB-03-HTB-1).
3.2.4. Nonlinearity

Material nonlinearity was included in the finite element analysis to predict the failure behavior of trabecular bone. Loading was generated in the model by prescribing a nominal strain of 2%. At such a low level of nominal strain, self-contact of the bone microstructure need not be considered (ABAQUS Technology briefs TB-03-HTB-1).

For low density trabecular bone, such as the human vertebra, tibia or greater trochanter, elastic buckling of the trabeculae may be responsible for collapse (Gibson, 1985; Muller et al., 1998; Stolken and Kinney, 2003). Under those circumstances, geometric nonlinearity (i.e. large deformation) is crucial to properly simulate the trabecular bone apparent level failure (Figure 3.2.5). It has been demonstrated that the geometric effects are minimal for high-density trabecular bone (Bayraktar et al. 2004a, 2004b). For normal rabbit distal femurs with high volume fractions like those used in this study (BV/TV>35%), plastic yielding should be the reason for bone failure and geometric nonlinearity is expected to be insignificant (Figure 3.2.6). However, for osteoporotic trabecular bone, the trabecular volume fraction may decrease significantly. In such a situation, geometric nonlinearity may play a more significant role in bone failure. To have the finite element models solved consistently and accurately, the geometric nonlinearity was considered throughout the study.
3.2.5. Boundary Condition and Related calculation

The frictionless boundary condition was used to simulate the lubricated platen mechanical test. A strain of 0.4% was used for linear analysis (without material and geometric nonlinearity) and a strain of 2% for studying yield (failure) behavior (with material and geometric nonlinearity).

When the load is applied in z direction, the apparent Young’s modulus in z-direction for the specimen as a whole is calculated by

\[ E'_z = \frac{\sigma^a_{zz}}{\varepsilon^a_{zz}} \]  \hspace{1cm} (3.2-2)

where \( \sigma^a_{zz} \) is the apparent stress and \( \varepsilon^a_{zz} \) the apparent strain in the z-direction. The apparent stress was calculated by

\[ \sigma^a_{zz} = F_r / (h_x h_y) \]  \hspace{1cm} (3.2-3)

where \( F_r \) is the total reaction force at the bottom face; \( h_x \) and \( h_y \) are specimen dimension size normal to the load direction. The apparent strain was calculated by
\[ \varepsilon_{zz}^{u} = \frac{u_p}{h_z} \]  
(3.2-4)

where \( u_p \) is the prescribed displacement; \( h_z \) is the specimen dimension size along the load direction. The yield strength was determined by the 0.2% offset method.

3.3. Bone tissue mechanical properties calibration

3.3.1. Specimen preparation

The accuracy of the heterogeneous finite element models partially depends on the fidelity of the mathematical equations with which the bone tissue material property is calculated. Though previous work has demonstrated the importance of heterogeneity in modeling trabecular bone (Bourne et al. 2004; Jaasma et al. 2002; van der Linden et al. 2001), only empirical or plausible correlations are available in the literature to capture the trabecular tissue heterogeneity. To have the trabecular bone finite element models render accurate and reliable results in this research, the trabecular tissue properties of the rabbit distal femur condyles were determined by an in house developed technique.

Twelve trabecular bone cubes with 4.5 mm dimension size in average (min 3.5 mm) were excised from the medial and lateral femoral condyles of 6-month old New Zealand White rabbits. The largest dimension of each specimen was controlled and oriented along the trabeculae direction (anterior-posterior). The specimens were scanned with 14 \( \mu \)m nominal resolution at 82kV and 97mA. After scanning, the apparent stiffness and strength of the cubes were determined by platen test along the anterior-posterior direction using the method described in this dissertation. Some values for the samples are shown in Figure 3.3.1.
The image stacks were degraded to 56 μm voxel size. Finite element models were then constructed from the degraded images using a direct voxel conversion technique. As the element size was much smaller than half the thickness of a trabecular strut (171 μm), it was assumed that the apparent moduli could be determined with sufficient accuracy (Harrigan et al., 1988; Homminga et al., 2001; Muller et al., 1996). Because the apparent modulus of trabecular bone is highly correlated to volume fraction (Rice et al. 1988), the degraded model’s volume fraction was forced to be the same as those of the original models by varying the threshold. The threshold values of the original models were determined by the method of Otsu (Otsu, 1979). This minimized the errors caused by
volume fraction variations. The non-bone elements and any loose or unconnected elements were simply eliminated from the models. The grayscales of the bone elements were converted to Hounsfield unit by Eq. 2.1-1. The bone tissue modulus for each element was then correlated to the corresponding Hounsfield unit. Along the anterior-posterior direction, the boundary condition was set to be frictionless on one surface and a 0.4% strain applied on the opposite surface for the bone tissue stiffness calibration and 2% for the strength calibration.

3.3.2. Material properties calibration technique description

Theoretically, bone tissue stiffness and strength can be calibrated within the same experiment. However, this combined calibration is extremely difficult because of computing limitation. The bone tissue stiffness was calibrated by linear analysis first and then the bone tissue strength by nonlinear analysis.

Both linear and power relations between trabecular bone stiffness and Hounsfield unit have been reported for apparent level trabecular bone studies (Keyak et al., 1994; Keller T.S., 1994; Kopperdahl et al., 2002; Rho et al., 1995). It is assumed that these correlations still hold on the bone tissue level and are related by:

$$E = a + b(NHU)^c$$  \hspace{1cm} (3.3-1)

where $NHU$ is the normalized Hounsfield unit (HU/10000) between 0 and 1. $a$, $b$, and $c$ are model parameters to be determined by a custom designed program written in Matlab v7.0 and Python v2.0. ABAQUS v6.5 was then called by the main program to run the analysis. The post-process was handled by a Python script because the ABAQUS output file is only accessible by either Python or C. For either power relation ($a=0$) or linear relation
(c=1), there were two parameters to be calibrated. As shown in the flow chart (Figure 3.3.2), all finite element models were solved with the program assigned bone tissue modulus within each iteration. The error function to be minimized is given

\[
I(a, b, c) = \frac{1}{n} \sum_{i=1}^{n} \left( \frac{k_i^{FEA} - k_i^{MTS}}{k_i^{MTS}} \right)^{a_{cbak}}
\]

where \( k \) is the apparent stiffness and \( i \) is number of models. The apparent stiffness of the trabecular samples was ranged from about 1 GPa to 3 GPa. The purpose of using percentage error rather than absolute error as the error function is to prevent the models being optimized from sacrificing the accuracy of the lower stiffness models. In addition, the error function had a single local minimum, which makes it more suitable to be a error function.

This 2-D minimization problem was solved by the Powell multidimensional minimization algorithm (Press et al. 2002). The minimization order was chosen as \((b, c)\) for the power correlation and \((a, b)\) for the linear correlation.
Since the bone tissue modulus-Hounsfield unit relationship may be corrupted by inaccurate specimens, it was necessary to refine the specimens and eliminate those that were damaged from the machining procedure. The apparent stiffness is different between the lateral and medial condyles (Bourne et al. 2004). In our experiment, the average trabecular apparent stiffness was 1.429 GPa (Std. Dev. 0.641 GPa) for the lateral condyles and 2.062 GPa (0.727 GPa) for the medial condyles (Figure 3.3.3). Two specimens from the medial group (5R and 6R) were significant softer than others and one (2L) was significant stiffer than others in the lateral group. A pre-calibration was performed by degrading the models to 84 μm element size. With this size of element, the calibration was finished quickly by sacrificing the accuracy. A power relation was acquired from this precalibrate with \( b=20609 \) and \( c=0.35 \). The FEA results (Figure 3.3.3)
show that the two suspected samples, 5R and 6R, had more than 40% errors (Figure 3.3.4, the average error of total twelve samples was 11%). Sample 2L in the lateral group was proven to be accurate. The two inaccurate samples with another one, which was too large for the whole calibration program to run efficiently, were eliminated from the calibration step but saved for later evaluation purpose. The coefficient of determination, also known as R-squared value is an indicator of how well the constructed model fits the actual data and thus was used to evaluate the quality of the calibrated model.

![Figure 3.3.3. Apparent stiffness of the twelve samples (FEA and MTS).](image1)

![Figure 3.3.4. Apparent stiffness percentage error of the twelve samples.](image2)
3.3.3. Strength calibration scheme

The nonlinear finite element analysis is much more time consuming than the linear analysis. Thus, an efficient calibration procedure requires choosing a proper correlation relationship with a minimum number of correlation parameters. For cortical and trabecular bone at the apparent level, it has been reported that the yield strength is linearly correlated to the stiffness (FyHrie and Vashisht, 2000; Rho et al., 1995; Yeni and Fyhrie 2001). This means the yield strain is the real parameter. Niebur et al. (2000) showed that trabecular bone has constant compressive and tensile yield strain. Similar results were also found for trabecular bone at the tissue level (Bayraktar et al 2004). The trabecular tissue was assumed to have a constant yield strain. Thus the bone tissue compressive yield strength was calculated by

$$
\sigma^c_y = e^c_y \times E
$$

(3.3-3)

where $E$ is the calibrated bone tissue Young’s modulus. Here only one parameter $e^c_y$ must be determined. Tensile yield strength is half of the compressive yield strength.

Eleven of the twelve specimens in the bone tissue stiffness calibration were used in the bone tissue strength calibration. The remaining sample was accidentally damaged, thus no strength information was recorded. The calibration was also performed in two categories, refined models (8 samples, without the 3 odd ones) and all models (11 in total). The experimentally tested yield strength was 24.364 MPa with standard deviation of 9.696 MPa for the refined group models. For the calibration procedure, the starting point was chosen as 0.0192 ($\sigma=0.0192 \times E$) which was determined from a coarse calibration. It took a total 492 CPU hours (around 10 days for two computers, Xeon 2.4
GHz processor and 2GB of RAM) for the eight models to complete one round of solutions.

3.3.4. Nonlinear analysis solving technique

It was observed that the computing cost would increase dramatically when refining the mesh for a trabecular bone model, which not only resulted from the increased DOFs but also the poorer convergence conditions. Some of the models even may not converge without a proper solution scheme. ABAQUS/standard uses Newton’s method by default to solve nonlinear models (Figure 3.3.5). For this method, the tangent stiffness, which is calculated from the end of the last iteration, is used in the current iteration. The tangent stiffness matrix is stored in a symmetric matrix by default even though there may be non-symmetric terms existing in the matrix. However, this default scheme was found to be both time consuming and poorly converging for the trabecular bone finite element models. ABAQUS recommends a quasi-Newton’s method for solving large finite element models. This technique was found to be efficient within iterations but needed many more iterations to converge than the standard Newton’s method. Because the unsymmetrical yield strength definition of the material properties resulted in unsymmetrical Jacobian matrix, when using the Newton’s method and unsymmetrical stiffness matrix, the trabecular bone models would converge within a reasonable number of iterations.
3.4. Accuracy of *in vivo* finite element models

Compared to *ex vivo* or *in vitro* micro-CT scans, *in vivo* micro-CT scans always have lower quality because of lower resolution and energy attenuation. The relatively poor quality of *in vivo* scans put the accuracy of corresponding finite element models into question. Thus before applying the *in vivo* finite element model to evaluate effects of osteoporosis, the accuracy of the *in vivo* model must be validated and the bias of the model, if any, must be determined and corrected. Twelve bone cubes were cut from six rabbit femurs. All cubes were physically tested and the finite element analyses were performed on the corresponding *in vivo* and *in vitro* scans to examine the accuracy of the calibrated heterogeneous bone tissue mechanical properties using the following schemes.
3.4.1. Accuracy determination scheme

The scheme of validating the accuracy of *in vivo* finite element models is shown in Figure 3.4.1. The micro-CT scans were divided into three categories. The *in vivo* scans were taken when the rabbit was still alive. The size of the rabbit leg with soft tissue limited the resolution of the *in vivo* scans. The *ex vivo* scans were made after the femur was excised from the rabbit leg. The scan quality was between the *in vivo* and *in vitro* scan. The *in vitro* scans in this research referred to the scans that were made on a bone sample (cube) which was cut from the condyle of rabbit distal femur. The *in vitro* scans had the best quality. After all three types of scans were made, the *in vivo* scans were registered to the *ex vivo* scans and then both of them were registered to the *in vitro* scans. After the registration, the trabecular cube was accurately located from the *in vivo* scans (Figure 3.4.1 registered scans column).
Figure 3.4.1. Accuracy of *in vivo* finite element models validation scheme.
Because of the extreme difficulty of directly registering the \textit{in vivo} scans to \textit{in vitro} scans, the \textit{ex vivo} scans served as an intermediate to bridge the \textit{in vivo} and \textit{in vitro} scans. The procedure of registering the \textit{in vivo} scans to the \textit{ex vivo} scans was relatively easy because the cortical shell of the distal femur could serve as a structural reference to assist the registration. The scheme of registering the \textit{ex vivo} scans to the \textit{in vitro} scans is described in Figure 3.4.2. Before making the \textit{ex vivo} scans, one cut was made perpendicular to the femur shaft as shown in step A. The cross-section area of the cut was kept intact during the following cutting procedure and this cross-section area was exactly one surface of the final cube. Thus this cross-section served as a reference plane. After the first cut, the \textit{ex vivo} scans were made with the slice parallel to the cross-section area (step B). The remaining five cuts were made after scanning (step D). The \textit{in vitro} scans were made after the cube was cut from the condyle and the slice was also parallel to the first cut as the \textit{ex vivo} scans (step E). This cutting scheme guaranteed that the first slice of the \textit{in vitro} scans was easily located from the \textit{ex vivo} scans as marked in light blue color in the first slice of \textit{ex vivo} scans in Figure 3.4.2. As the rough location of the cube in the \textit{ex vivo} scans was located, the following registration procedure was straightforward. With the help of \textit{ex vivo} scans, finally the \textit{in vivo} scans were registered to the \textit{in vitro} scans. In the column of registered scans in Figure 3.4.1, a slice of each cube from the three types of scans is displayed. The quality difference between different types of scans is obvious from these images. Finite element models were then constructed from the \textit{in vivo} scans and nonlinear analysis was performed. The corresponding trabecular cube was then positioned on the MTS machine and loaded to fail. By comparing the stiffness and
strength acquired from both methods, the accuracy and the bias, if any, could be 
determined.

In addition to the heterogeneous models, the corresponding homogeneous models 
were also constructed and solved to demonstrate the advantage of heterogeneous models. 
The homogeneous models were exactly the same as heterogeneous models except the 
material stiffness and strength definition. The homogeneous material properties were 
calibrated separately with the same procedure as heterogeneous material properties. 
However, because there is only one number for bone tissue stiffness other than an 
equation, the calibration procedure is much faster.
Figure 3.4.2. Technique description for \textit{in vitro} scans being registered to \textit{ex vivo} scans.
3.4.2. Volume fraction correction

The *in vitro* and *ex vivo* scans were all made at 82keV and 97mA (the standard setup defined in this research) whereas the *in vivo* scans were made at 75kV and 107mA. The method illustrated in Chapter 2.5.2 was used to correct this inconsistent power setup to acquire correct image Hounsfield unit.

Regarding the same group of specimens, when applying the Otsu method to determine the trabecular volume fraction (BV/TV) for the *in vivo* and *in vitro* scans separately, the *in vivo* scans had an average volume fraction 4.3% higher (std. dev. 1.6%) than the *in vitro* scans. Because the bone volume fraction can significantly affect the corresponding mechanical properties determined by FEA, and because the bone tissue properties were calibrated by *in vitro* trabecular bone specimens, it was necessary to correct this inconsistency of volume fraction. The volume fractions calculated from the *in vitro* models are plotted against those calculated from the *in vivo* models (Figure 3.4.3). The biases consistently follow a trend line with $R^2$ value of 0.98. Thus the trend line given by

$$VF_{in vitro} = 1.1697 * VF_{in vivo} - 0.1193$$ \hspace{1cm} 3.4-1

was used to correct the volume fraction errors of the *in vivo* models.
3.5. Effect of ovariectomy in terms of mechanical properties evaluated by nonlinear heterogeneous finite element analysis

3.5.1. Experiment design

One purpose of this research is to noninvasively evaluate trabecular bone micro-architectural changes in terms of apparent stiffness and strength. This evaluation was performed using micro-CT during the aging of normal rabbits and rabbits treated with ovariectomy for the purpose of inducing osteoporosis.

A total of six mature non-pregnant female New Zealand white rabbits were used. After growth plate closure (age six months), three of the rabbits were bilateral ovariectomized (OVX group) and the rest were sham operated (INTACT group) with full exposure of both ovaries prior to surgical closure.

While still under sedation, all six rabbits underwent micro-CT scanning of the right distal femur; this is referred to as day 0 (D0). After 91 days (D91), the rabbits were
scanned again over the same region as the scans made on D0. The scan region is shown in Figure 3.5.1.

Figure 3.5.1. *In vivo* scan on the same day as surgery (Day 0) and 91 days following (Day 91).

Care was taken to make the two scans of a common rabbit at roughly the same region to minimize the effort needed for image registration. Finite element models of the rabbits distal femoral condyles were divided into four groups (Figure 3.5.2). Statistical analyses were performed between groups and within groups to evaluate the ovariectomy effect on trabecular bone growth.

<table>
<thead>
<tr>
<th>In vivo scan</th>
<th>OVX Group</th>
<th>INTACT Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>FEA</td>
<td>FEA</td>
</tr>
<tr>
<td>Day 91</td>
<td>FEA</td>
<td>FEA</td>
</tr>
</tbody>
</table>

![Diagram showing intra and inter group comparison](image)

Figure 3.5.2. Statistical analyses were performed between groups and across time points.
3.5.2. Building and solving finite element models

After registration of the scans on D0 and D91, twenty-four 4 mm\(^3\) trabecular bone cubes were cut from both condyles of all distal femur scans. The cubes were cut in the condyle region where the trabecular web was dense and relatively uniform as indicated in Figure 3.1.2. Care was taken to cut the cube following the anterior-posterior direction of the trabecular bone. The registration and the program controlled cutting procedure guaranteed that the cubes on scans D0 and D91 were exactly within the same region of interest (ROI). In Figure 3.5.3, two cubes from D0 and D91 respectively show very similar patterns, which demonstrate the validity of following statistical analyses with respect to the FEA results. A randomly selected slice from the cube D0 with the corresponding slice of cube D91 are shown in Figure 3.5.4 which further shows the quality of registration by the similar pattern in 2D images.

Figure 3.5.3 Cubes cut from the scans of D0 (left) and D91 (right) from the same rabbit. Registration and program controlled cutting procedure resulted in two cubes on the same ROI.
The procedures of creating the finite element models and ABAQUS input files together with the registration procedure are shown in the flow chart of Figure 3.5.5. The step-by-step sequence with the methods or theories used for each step and the program to accomplish each step are demonstrated in this flow chart. Following image registration and cube cutting, the voxel size was degraded using the voxel expansion (VE) method to 56 µm from 28 µm, the original resolution. The cube image stacks were then segmented.

To prevent the volume fraction inconsistency between the original scans and the degraded scans affecting the subsequent finite element analysis, the degraded 56 µm images were forced to have the same volume fraction as the original ones, the threshold values of which were automatically determined by the method of Otsu. After segmentation, the grayscales below the threshold were all eliminated. Those above the threshold were retained and used to calculate the heterogeneous bone tissue material properties. Following segmentation, unconnected voxels were eliminated. The image...
degradation, segmentation and unconnected voxel elimination were categorized as pre-mesh steps and accomplished in a Matlab program (Premesh.m).

The next step was to convert the image stacks into finite element models by the voxel conversion technique in which each voxel was converted to an 8-node brick element. For each element (voxel), the grayscale value was converted to its corresponding Hounsfield unit by comparing it with the water and air phantom grayscale values using Eq. 2.1.1. Because of the CT machine’s problem, some scans were made under nonstandard power setup to acquire best image quality, correction was made to avoid any error induced by incorrect Hounsfield unit calculation. Following the rules of the ABAQUS script, a node file was generated by recording the nodes’ spatial locations. An element file was created to record all elements definitions. A node set file and an element set file were created separately to store all necessary node set (spatial) and element set (property) information. The primary node sets included the LOADSET which recording the nodes that the load was applied on and the BOUNDSET on which the boundary condition was applied. There were also some other node sets to assist in simulating the frictionless boundary condition and post-process calculation. The elements with the same tissue properties were grouped in one element set. These node set or element set files only record the geometry information of the models; thus, auxiliary files were created independent of the following main input file where material properties and all necessary control information of the FE model were stored. The advantage of this scheme is that the parameter controlled main file could be modified as user required while the auxiliary files are kept unchanged. These three steps (image to FEM conversion, Hounsfield unit conversion and ABAQUS
file (Geometry information) generation) were accomplished by the Matlab program named MeshCT.m.

Figure 3.5.5. Finite element models generation procedure flow chart.
Young’s modulus and stress given by Eq 3.5-1 were input to para_strength.m, the main input file was generated and ready to run in ABAQUS.

\[
E(\text{MPa}) = 1.65E4(\text{NHU})^{0.23} \\
\sigma(\text{MPa}) = 0.0199E
\]

A sample ABAQUS main input file (tra_model.inp) is shown in Appendix A with a detailed comment (comment line is led by ** mark).

Two workstations were used to solve the models. One workstation has 2 GB RAM with dual Xeon 2.4 GHz CPUs. Another one has 2 GB RAM (upgraded to 2.5 GB later) with single Xeon 2.4 GHz CPU. To avoid the 2 GB virtual memory limitation per process in Windows XP system, the 3 GB switch was turned on to have those large models run properly. The solution time varied from approximately 12 hours to 2 - 3 days; this largely depended on model size (number of elements), maximum wavefront value and convergence condition.

A program written in Python read the necessary data out from the ABAQUS ODB files (result file). The data was forwarded to the power_strength.m program to calculate the models apparent stiffness and strength using the 0.2% offset method. Thus the whole procedure, from preprocessing of model generation, meshing, solving, until the step of postprocessing, was done independent of ABAQUS GUI mode and no user effort was required (Figure 3.5.6)
Figure 3.5.6. Trabecular models solving scheme
CHAPTER 4. RESULTS

In this chapter, the bone tissue stiffness and strength calibration results will be presented, followed by the finite element model accuracy validation results. These results demonstrate that the methods and techniques in performing finite element analysis on trabecular bone samples were feasible and the corresponding data were accurate. Finally, the experimental results for using nonlinear heterogeneous finite element models to evaluate trabecular bone osteoporosis were demonstrated.

4.1. Calibration results

4.1.1. Bone tissue stiffness calibration results

The 9 calibration models were run on a workstation with two Xeon 2.4 GHz processor and 2 GB of RAM. The 9 models took 3.4 hours to finish one iteration of the optimization procedure. The best fit power correlation was achieved using

\[ E(GPa) = 16.54(NHU)^{0.23} \]  

where the corresponding error function value \( I \) (Eq. 3.3-2) was 2.86%. The variation of error function value \( I \) vs. power \( c \) is shown in Figure 4.1.1. As \( c \) decreased from 1.0, \( I \) also decreased until reaching a minimum at \( c=0.23 \). After that, \( I \) increased until \( c=0 \); at this point, the heterogeneous models became homogeneous models. A comparison plot of FEA and MTS moduli values is shown in Figure 4.1.3. The \( R^2 \) value for the refined models was 0.987 (for all twelve models 0.867). Figure 4.1.4 shows the FEA-MTS errors...
are plotted on the Y-axis vs. the MTS results, considered to be the true values, plotted on the X-axis. For the refined models, the standard deviation (87.34 MPa) and average (-30.08 MPa) of stiffness errors proved the accuracy of the calibration. The bone tissue modulus from the power correlation ranged from 9.84 GPa (std. dev. 0.204) to 13.89 GPa (std. dev. 0.456). The coefficient of variation (COV= standard deviation/mean) is 6.4% (std. dev. 0.57%).

![Figure 4.1.1. The error function value $I$ vs. power $c$.](image1)

![Figure 4.1.2. Curves for best fitted linear and power correlation. The horizontal line is for the homogeneous models.](image2)

![Figure 4.1.3. Apparent stiffness from power correlation (FEA vs. MTS)](image3)

![Figure 4.1.4. Apparent stiffness from power correlation (agreement testing)](image4)
For the linear correlation equation, the final calibrated equation was

\[ E(\text{GPa}) = 12.1 \times (\text{NHU}) + 8.932 \]  

(4.1-2)

The corresponding curve of this equation is shown in Figure 4.1.2. The minimum error function value \( I \) was 2.89\% which is slightly larger than that of the power function 2.86\%. The \( R^2 \) is 0.986 for refined models and 0.867 for all models (Figure 4.1.5). The agreement testing is shown in Figure 4.1.6. Both Figure 4.1.5 and Figure 4.1.6 demonstrate a similar quality as the power correlation. For this linear correlation, the bone tissue modulus was ranged from 10.2 GPa (std. dev. 0.116) to 14.49 GPa (std. dev. 0.675). The COV is 6.2\% (std. dev. 0.48\%).

For the power correlation \( E=b(\text{NHU})^c \) with \( c=0 \) or the linear correlation \( E=a+b(\text{NHU}) \) with \( b=0 \), the bone tissue stiffness \( E \) became a constant number. Accordingly, the heterogeneous finite element model became homogeneous. The error function value \( I \) for the best fit homogeneous models was 3.04\%. Corresponding
homogeneous bone tissue modulus was 11.768 GPa (Figure 4.1.2). Compared to the $I$ value (2.86%) of the best fit power equation, the homogeneous model had 0.18% increase in the average errors. The $R^2$ value of the homogeneous model was 0.986, which was as good as that of the heterogeneous models.

The linear equation and power equation have such similar correlation that either of them can be used. Because it is more often employed in the literature, the power equation was chosen for this study.

A number of previous finite element analyses on the trabecular bone modulus have been performed on human vertebra, human femur, and bovine tibia (Mente et al. 1989, Hou et al. 1998, Ladd et al. 1998a, Rüegsegger et al. 1996). In these studies, homogeneous moduli were assumed and a range of tissue modulus from 5.7 Gpa (std. dev. 1.6 GPa) to 17.3 GPa (std. dev. 2.62 GPa) were determined. Using other techniques, this number could range from 2.11 GPa (std. dev. 1.89) (Riemer et al. 1994) to 18.14 GPa (std. dev. 1.7) (Turner, et al, 1999). The bone tissue stiffness from the study ranged from 9.45 GPa to 14.87 GPa which was well fell within the range of these previous studies.

4.1.2. Bone tissue yield strength calibration results

The strength calibration procedure is similar to the stiffness calibration except that there was only one parameter $\epsilon^c_y$ that must be calibrated. However, because of the essence of nonlinear analysis, it was much more time consuming than the stiffness calibration. The calibration procedure stopped when the variation of strain was within 0.02%. The error function reached its minimum when $\epsilon^c_y = 0.0199$. The FEA strength was plotted against the MTS results in Figure 4.1.7. The $R^2$ value was 0.957 for the refined models and 0.877 for all models. From the agreement testing (Figure 4.1.8), the mean
error was -0.039 MPa, and the standard deviation was 2.35 MPa for the refined models.
For all models, the corresponding values were 1.098 MPa and 3.26 MPa.

The apparent stiffness results from the nonlinear FEA were also evaluated and displayed in Figure 4.1.9 and Figure 4.1.10. For the refined models, the averaged error was -24.17 MPa with standard deviation of 80.60 MPa. The corresponding R-squared value was 0.988. For all models, the error mean and standard deviation were 96.50 MPa and 267.61 MPa with the R-squared value equal to 0.87.
The apparent stiffness calculated from the nonlinear analysis was close to that from the linear analysis with p-value of 0.98 for a two-tail paired t-test. Compared to linear analysis, the nonlinear analysis considered the material nonlinearity and geometric nonlinearity (Buckling). However, before the yielding point, the material nonlinearity did not contribute significantly to the model deformation. Buckling is the primary concern that can make the nonlinear analysis significantly differ from linear analysis within the linear deformation domain. The t-test result indicated that the buckling effect was probably insignificant for the trabecular bone models with a relatively high average bone volume fraction of 0.373. Thus the bone tissue stiffness calibrated from the linear analysis could safely be used in nonlinear analysis without any bias with geometry nonlinearity (buckling effect) being considered.

The calibration results demonstrated that the trabecular bone tissue heterogeneity could be accurately determining by large scale finite element models and mechanical experiment. By correlating the bone tissue stiffness and strength to bone tissue Hounsfield unit, the nonlinear heterogeneous finite element models were able to predict the apparent stiffness and strength accurately.

4.2. *In vitro* and *In vivo* finite element model accuracy determination

Prior to validating the *in vivo* models, the *in vitro* finite element models were constructed and analyzed first to confirm the calibrated bone tissue correlation equations. The apparent stiffness and strength from nonlinear heterogeneous finite element models were compared to those of the mechanical experiments. The apparent stiffness and
strength of *in vitro* finite element models results are displayed in Table 4.2.1. Figure 4.2.1 and Figure 4.2.2 demonstrate the correlation of the FEA results to the MTS results.

### Table 4.2.1 *In vitro* heterogeneous models error statistics results.

<table>
<thead>
<tr>
<th>Heterogeneous models</th>
<th>Error Mean (MPa)</th>
<th>Error Std. Dev (MPa)</th>
<th>FEA-MTS R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent Stiffness</td>
<td>35.95</td>
<td>271.4</td>
<td>0.7702</td>
</tr>
<tr>
<td>Apparent Strength</td>
<td>2.127</td>
<td>4.623</td>
<td>0.7592</td>
</tr>
</tbody>
</table>

Figure 4.2.1. Apparent stiffness from the *in vitro* cube finite element models vs. that of the mechanical experiments.

Figure 4.2.2. Apparent strength from the *in vitro* cube finite element models vs. that of the mechanical experiments.

The corresponding homogeneous models were also solved with the calibrated bone tissue stiffness 11.768 GPa and the same bone tissue stiffness and strength relation. The statistical error results are shown in Table 4.2.2

### Table 4.2.2 *In vitro* homogeneous models error statistics results.

<table>
<thead>
<tr>
<th>Homogeneous models</th>
<th>Error Mean (MPa)</th>
<th>Error Std. Dev (MPa)</th>
<th>FEA-MTS R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent Stiffness</td>
<td>135.2</td>
<td>295.7</td>
<td>0.6712</td>
</tr>
<tr>
<td>Apparent Strength</td>
<td>3.956</td>
<td>5.858</td>
<td>0.5296</td>
</tr>
</tbody>
</table>

The *in vivo* finite element models of the trabecular cubes were constructed and analyzed. The statistical errors are displayed in Table 4.2.3. The apparent stiffness results
from FEA versus MTS are plotted in Figure 4.2.3 and the apparent strength from FEA versus MTS is plotted in Figure 4.2.4.

<table>
<thead>
<tr>
<th>Heterogeneous models</th>
<th>Error Mean (MPa)</th>
<th>Error Std. Dev. (MPa)</th>
<th>FEA-MTS R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent Stiffness</td>
<td>-69.84</td>
<td>288.6</td>
<td>0.7286</td>
</tr>
<tr>
<td>Apparent Strength</td>
<td>0.248</td>
<td>3.347</td>
<td>0.8968</td>
</tr>
</tbody>
</table>

It was expected that the \textit{in vitro} finite element models would show a relative lower R-squared values (0.7702 for apparent stiffness and 0.7592 for apparent strength) than those of the Chapter 4.1.2 where refined models were used and all R-squared values were greater than 0.8. Because there was no refinement for the current analysis, all possible error sources can contribute to the relatively low R-squared values. The primary sources of error include the bone cutting procedure, where micro-damage might be caused by the diamond saw; the loading procedure, where unparallel compression surfaces may significantly lower the sample stiffness and strength; the quality of the micro-CT scans might also be a factor.
The error function, $I$, used in the bone tissue stiffness calibration was to minimize the average percentage error (Eq. 3.3.2). This design of error function was intended to allow smaller errors for the lower apparent stiffness models while relative larger errors for the models with higher apparent stiffness. The effect of this design is reflected in Figure 4.2.1 and Figure 4.2.3, where the FEA errors tend to increase as the apparent stiffness increases. Also the average error of apparent stiffness (35.949 MPa) and strength (2.127 MPa) were all positive, which means the material properties calibrated based on Eq. 3.3.2 resulted in a slightly stiffer modulus than actual ones. Compared to the in vitro models, the in vivo finite element models were softer with -69.84 MPa average errors for stiffness and 0.248 MPa for strength prediction. The reasons of the softening results could result from the corrected volume fraction. When creating the in vivo finite element models, the volume fraction of the bone models were higher than the corresponding in vitro models. Thus correlation equation 3.4-1 was used to increase the threshold value to force the in vivo models to have the same volume fraction as the in vitro models. However, this volume fraction correction brought in a side effect that some trabecular connections would be broken during the correction procedure which resulted in lower stiffness and strength in the in vivo finite element models. However, this softening property of in vivo models countered the calibrated slightly stiffer modulus, which made the in vivo FEA results being as good as or even better than the in vitro results.

From the results shown in Figure 4.2.3 and Figure 4.2.4, it can be concluded that the in vivo finite element models can predict the apparent strength and stiffness with acceptable accuracy.
The corresponding homogenous models were also solved to prove the advantages of the heterogeneous models for the *in vivo* analysis. The statistical FEA results are shown in Table 4.2.4. Homogeneous models also showed a very good prediction of the apparent stiffness and strength. However, compared with these results, the heterogeneous models, which had R-square values of 0.7286 for apparent stiffness and 0.8968 for apparent strength, are better in predicting the trabecular bone apparent properties.

<table>
<thead>
<tr>
<th>Homogeneous models</th>
<th>Error Mean (MPa)</th>
<th>Error Std. Dev. (MPa)</th>
<th>FEA-MTS R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent. Stiffness</td>
<td>-151.8</td>
<td>311.4</td>
<td>0.626</td>
</tr>
<tr>
<td>Apparent. Strength</td>
<td>-0.524</td>
<td>3.617</td>
<td>0.878</td>
</tr>
</tbody>
</table>

### 4.3. Osteoporosis evaluation results

#### 4.3.1. Volume fraction results

As the trabecular bone apparent stiffness is highly correlated to the trabecular bone volume fraction, the volume fraction was first analyzed to establish a reference to which the FEA results could be compared. For the purpose of consistency, the light grey color will be used to indicate the INTACT group, while the white color for the OVX group for all the bar charts in this chapter.

Figure 4.3.1 and Figure 4.3.2 demonstrate the volume fraction comparison between the OVX and INTACT groups. On Day 0 (D0), the cubes had similar volume fraction for both lateral (p=0.709 for t test. Same as following) and medial (p=0.761) groups which indicated that there was no induced bias at the start point of the experiments between groups. On Day 91 (D91) after three months, the difference between the OVX and INTACT groups became obvious. Though the p values from one-tail test, 0.062 for the
lateral condyles and 0.138 for the medial ones, do not qualify for a significant difference ($\alpha=0.05$), they did approach the number of 0.05. More information could be acquired from another inner group comparison. For the OVX group (Figure 4.3.3), the volume fraction was decreased 0.91% for the lateral condyles and increased 2.15% for the medial condyles. Neither showed any significant difference after three months period ($p=0.927$ and 0.805 for lateral and medial respectively). While for the INTACT group (Figure 4.3.4), the volume fraction increased 11.73% ($p=0.058$, approaching 0.05) and 9.17% ($p=0.026$) in average for lateral and medial condyles respectively. This discrepancy between the OVX and INTACT groups reflected that ovariectomy did slow down or prohibit the trabecular bone from becoming denser.

![Graph](image1)

**Figure 4.3.1**, Lateral condyle same day comparison ($p=0.709, 0.062$).

![Graph](image2)

**Figure 4.3.2**, Medial condyle same day comparison ($p=0.761, 0.138$).

![Graph](image3)

**Figure 4.3.3**, OVX group condyle within category comparison ($p=0.927, 0.805$).

![Graph](image4)

**Figure 4.3.4**, INTACT group condyle within category comparison ($p=0.058, 0.026$).
4.3.2. Apparent strength analysis

It was shown that for the lateral condyles on D0 (Figure 4.3.5), the samples had an apparent strength average of 24.00 MPa (9.04 MPa) for the OVX group and 27.37 MPa (3.75 MPa) for the INTACT group. Statistically, there was no significant difference (p=0.583) found between these two groups. On the D91 of the experiment, the strength was 27.64 MPa (10.91 MPa) for the OVX group and 40.86 MPa (0.73 MPa) for the INTACT group. The p value of 0.086 (one-tail) indicated that a significant difference was approached between two groups as time went on. A similar statistics analysis results were also observed for the analysis of medial condyles (Figure 4.3.6). On the first day, the average strength was 40.24 MPa (12.07 MPa) and 39.43 MPa (4.85 MPa) for OVX and INTACT group respectively (p=0.919). On the last day of the experiment, the strength of INTACT group, 57.66 MPa (2.25 MPa), significant surpassed the strength of the OVX group 45.48 MPa (12.06 MPa) (p=0.114).

For the inner group analysis of the OVX group (Figure 4.3.7), the strength variations were 15.15% (p=0.340) and 13.04% (p=0.311) in average for the lateral and medial condyles respectively. The results for the INTTACT group showed significant for both condyles (p=0.013, 0.002). The strength had a 49.30% and 46.23% increase for lateral and medial condyles respectively.
Figure 4.3.5, Lateral condyle inter-group same day comparison, P=0.583, 0.086.

Figure 4.3.6, medial condyle inter-group same day comparison, P=0.919, 0.114.

Figure 4.3.7, OVX intra group comparison. P=0.340, 0.311. Average strength difference regarding Fday-Lday comparison is 15.15% and 13.04% for lateral and medial condyles respectively.

Figure 4.3.8, INTACT intra group comparison. P=0.013, 0.002. Average strength difference regarding Fday-Lday comparison is 49.30% and 46.23% for lateral and medial condyles respectively.

4.3.3. Apparent stiffness analysis

The same statistics analysis was also performed on the apparent stiffness results. For the inter group comparison, the apparent stiffness results demonstrated very similar statistic results to that of the strength analysis (Figure 4.3.9, Figure 4.3.10). In Figure 4.3.11, the OVX analysis showed that the lateral condyles apparent stiffness had a 9.00% increase from 1.82 GPa (0.6 GPa) to 1.98 GPa (0.67 GPa) on average. The medial condyles had an 11.49% increase from 2.84 GPa (0.70 GPa) to 3.16 GPa (0.69 GPa). However these increases were not significant base on a t-test analysis (p=0.769, 0.598). For the INTACT group (Figure 3.1.12), the apparent stiffness had an average of 40.08%
and 34.18% increase for the lateral and medial condyles. The p values of 0.022 and 0.008 showed significant difference between the D0 and D91 apparent stiffness.

<table>
<thead>
<tr>
<th>Scan Time (day)</th>
<th>Stiffness (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>1000</td>
</tr>
<tr>
<td>D91</td>
<td>2000</td>
</tr>
<tr>
<td>OVX</td>
<td></td>
</tr>
<tr>
<td>INTACT</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.3.9, Lateral condyle inter-group same day apparent stiffness comparison, P=0.632, 0.081

<table>
<thead>
<tr>
<th>Scan Time (day)</th>
<th>Stiffness (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>1000</td>
</tr>
<tr>
<td>D91</td>
<td>2000</td>
</tr>
<tr>
<td>OVX</td>
<td></td>
</tr>
<tr>
<td>INTACT</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.3.10, Medial condyle inter-group same day apparent stiffness comparison, P=0.996, 0.129

<table>
<thead>
<tr>
<th>Lateral Medial Condyle Category</th>
<th>Stiffness (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td></td>
</tr>
<tr>
<td>D91</td>
<td></td>
</tr>
<tr>
<td>OVX</td>
<td></td>
</tr>
<tr>
<td>INTACT</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.3.11, OVX inner group comparison, P=0.769, 0.5978. Average stiffness difference regarding Fday-Lday comparison is 9.00% and 11.49% for lateral and medial condyles respectively.

<table>
<thead>
<tr>
<th>Lateral Medial Condyle Category</th>
<th>Stiffness (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td></td>
</tr>
<tr>
<td>D91</td>
<td></td>
</tr>
<tr>
<td>OVX</td>
<td></td>
</tr>
<tr>
<td>INTACT</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.3.12, INTACT inner group comparison, P=0.022, 0.008. Average stiffness difference regarding Fday-Lday comparison is 40.08% and 34.18% for lateral and medial condyles respectively.

The FEA results showed that the apparent strength and stiffness of the INTACT group rabbits increased significantly during the three months. On the other hand, only limited and insignificant increases were observed for both strength and stiffness analysis for the OVX group (Figure 4.3.7, Figure 4.3.11). These results validated the expected ovariectomy effect which was not very obvious from the volume fraction analysis.
4.3.4. Bone tissue properties analysis

The variation of the trabecular bone tissue properties was also examined to determine the potential ovariectomy effect at the tissue level. For each model, the bone tissue stiffness was averaged throughout the model. This averaged bone tissue stiffness was pooled together for all models regarding the OVX or INTACT group. The OVX analysis (Figure 4.3.13) showed that the lateral group bone tissue stiffness increased from 13.13 GPa (Std. Dev. 0.12 GPa) to 13.84 GPa (Std. Dev. 0.54 GPa). The medial group increased from 13.20 GPa (Std. Dev. 0.18 GPa) to 13.95 GPa (Std. Dev. 0.37 GPa) in the mean time. For the INTACT group (Figure 4.3.14), the averaged bone tissue stiffness increased from 13.16 GPa (Std. Dev. 0.1 GPa) on to 13.76 GPa (Std. Dev. 0.36 GPa) for the lateral condyles. For medial condyles, the bone tissue stiffness varied from 13.09 GPa (Std. Dev. 0.11 GPa) to 13.79 GPa (Std. Dev. 0.43 GPa). All these increases were significant based on the p values.

Figure 4.3.13, Bone tissue stiffness increased for OVX group. P=0.045 and 0.017 for lateral and medial condyles respectively.

Figure 4.3.14, Bone tissue stiffness increased for INTACT group. P=0.025 and 0.027 for lateral and medial condyles respectively.
Conflicting data exists regarding the osteoporotic bone tissue composition; some studies report that the mineral content is unchanged or slightly lower during osteoporosis (Kasugai et al., 1998; Yoshitake et al., 1999) where others report an increase in the mineral content and a lack of collagen (Birkenhaeger et al., 2000; Boyde et al., 1998; Zioupos and Aspden, 2000). McNamara et al. (2006) also observed an increase in mineral content of bone tissue which confirmed their findings of increased mechanical properties at the level of the bone trabeculae. For the current research, as shown in Figure 4.3.15, where the amount of tissue stiffness increase is demonstrated, it was found that the bone tissue stiffness of the OVX group had a slightly higher increase than the INTACT group during the same time span. Because the bone tissue strength was correlated to tissue stiffness with a constant yield strain of 0.0199, the bone tissue strength variation was expected to have the same statistic analysis results as tissue stiffness. This bone tissue level mechanical properties analysis indicated that ovariectomy had a limited, if any, effect on tissue level properties.

Figure 4.3.15, The amount of bone tissue stiffness increase for the OVX and Intact groups
4.3.5. Homogeneous models results

The homogeneous models were also solved. The bone tissue stiffness was set to be a constant of 11.768 GPa which was calibrated in the tissue properties calibration section. Previous results showed that the increase of apparent stiffness and strength were observed for both the OVX and INTACT groups. The increase in percentage was also examined from the homogeneous analysis. The results from both homogeneous and heterogeneous models are displayed in Table 4.3.1. Consistently lower percentage increases were observed for the homogenous models compared to the heterogeneous ones. Considering the results shown in Figure 4.3.15, because the homogeneous models could not capture the tissue level material properties variations, it is easy to understand why the homogeneous models predicted a low apparent stiffness and strength increase than the heterogeneous models.

<table>
<thead>
<tr>
<th></th>
<th>Strength</th>
<th>Heterogeneous</th>
<th>Homogeneous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OVX</td>
<td>Lateral 15.15%</td>
<td>7.42%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medial 13.04%</td>
<td>6.40%</td>
</tr>
<tr>
<td>INTACT</td>
<td>Lateral 49.30%</td>
<td>38.48%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medial 46.23%</td>
<td>35.97%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Stiffness</th>
<th>Lateral 9.00%</th>
<th>2.05%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OVX</td>
<td>Medial 11.49%</td>
<td>5.09%</td>
</tr>
<tr>
<td>INTACT</td>
<td>Lateral 40.08%</td>
<td>30.15%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medial 34.18%</td>
<td>24.83%</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.3.1, Increase percentage comparison for results from homogeneous and heterogeneous models.
CHAPTER 5. DISCUSSION

5.1. Significance of the research

It is common to differentiate the factors that determine bone strength into quantity, expressed as bone mass or density, and quality, as represented by its trabecular architecture and the characteristics of the mineralization matrix (Chavassieux et al., 1996). It was found that 60-70% of the strength could be explained by density (Goulet et al., 1994). Thus 30-40% of trabecular bone strength depends not on the quantity of bone, but on the quality of the trabecular architecture and that of the mineralized tissue. For stiffness, this is about 20-40% (Bouxsein et al., 1996). Similar results have been demonstrated using the data for bone tissue properties calibration where both mechanical experiment and FEA results were available. In Figure 5.1.1 and Figure 5.1.2, it has been shown that the volume fraction could explain 74.31% of the apparent strength and 80.43% of the apparent stiffness where the mechanical experiment results were taken as the true values. In Figure 5.1.3 and Figure 5.1.4, it was demonstrated that the finite element analysis method could explain 90.39% of the apparent strength and 88.3% of the apparent stiffness. Thus the results showed that the FEA method could explain the trabecular bone mechanical properties 10-20% higher than the volume fraction alone did.
Thus the advantage of finite element analysis is that it can account for both the bone quantity and the bone architecture and possible mineral quality changes. For homogeneous models, the bone tissue properties, which numerically represent the mineral qualities, are constant. It is incapable of capturing the mineralization variations. The heterogeneous models, on the other hand, reflect the mineral quality change by relating the bone tissue properties with the bone tissue density. Thus any mineral quality changes in trabeculae could be reflected in the finite element model’s tissue definition and in turn affect the model’s apparent level mechanical behavior. It has been shown that the homogeneous bone tissue properties were carefully calibrated with an R-squared
value comparable to that of the heterogeneous models (Figure 4.1.2). However, when applying these calibrated mechanical properties on another group of trabecular bone models, homogenous models showed a less accuracy than the heterogeneous models (Table 4.2.1-Table 4.2.4). These calibrated bone tissue properties also resulted in a consistent lower prediction of the apparent mechanical properties increase (Table 4.3.1).

One possible reason for this discrepancy is the less accurate homogeneous model which lacked the capability of representing the bone tissue property variation. For the OVX group, the homogeneous models only monitored half or even less of the apparent properties increase than the heterogeneous models (Table 4.3.1). These facts demonstrate the advantage of using heterogeneous finite element models to simulate trabecular bone physical behavior, especially to monitor the mechanical properties variations during a time span.

Trabecular bone strength is the most direct way to assess osteoporosis. Traditionally for simplicity, either volume fraction with other auxiliary indices or apparent stiffness was evaluated to estimate the apparent strength. To do so, an equation must be constructed to evaluate the strength from stiffness or volume fraction. Yet these equations are subjected to change when the target samples change, which makes the method unreliable to some extent. In the current research, nonlinear finite element models were used to evaluate the strength more directly. The nonlinear models consider both material nonlinearity and geometry nonlinearity (buckling), which makes the models versatile and reliable for varieties of samples.

By using the \textit{in vivo} scanning technique, accurate finite element models were able to evaluate the trabecular bone mechanical properties non-invasively. Further more, by
using the image registration technique, the mechanical property variations at any two or more time points could be accurately and easily determined. Thus it provides a useful tool to monitor or assess osteoporosis. The potential application of these techniques on humans will improve the assessing of osteoporosis.

In addition, this research is the first attempt to determine the trabecular tissue properties by implementing a so called back calculation method at the tissue level with multiple parameters involved.

This research developed a reliable routine from the image processing to trabecular finite element model construction and analysis. It provided techniques that address all possible problems encountered in this procedure. It also suggested the most feasible solver to perform the nonlinear analysis considering both efficiency and convergence. The procedure was highly program controlled and provided the user flexibility as well.

For application purpose, this work was compared with that of Pistoia et al. (2002). They employed the method which determined the failure status by counting the yielded elements. In their method, linear homogeneous models were used to model the human distal radius. A predefined load was applied and the models were solved. After solution, they linearly scaled the element strains and the applied load. When the amount of elements exhibiting yield behavior reached 2%, the model was considered to be at a yield condition and the corresponding load was the failure load. Compared to the mechanical experiment, the R-square value of the correlation was up to 75%. However, it was also found that the prediction overestimated the actual fracture load up to 29%. One of the sources of this overestimation may be that the models lacked the ability to capture the buckling effect which would affect the model behavior. Our heterogeneous finite element
models showed up to 90% R-square values when predicting the in vivo models’ strength. No significant overestimation or underestimation trend was observed. Geometric nonlinearity was considered to capture the buckling effect of the model. Without any error-canceling effect, a high R-square value still could be guaranteed. Compared to the work of Pistoia et al. (2002), the complex solving procedure and high cost are the main limitations of the current approach. Thus their method has advantage in solving large models, such as whole bone modeling. Our model is more accurate in solving moderate size models such as the trabecular bone cube models used in this dissertation.

5.2. Limitation and possible improvement

The increase, rather than a decrease, of the trabecular bone mechanical properties in the OVX group was different from what was expected in this research. Two possible reasons may have caused this increase. First, the six-month old rabbits were supposed to be skeletally mature. Yet from the INTACT group, nearly 50% of increase in strength was observed in the following three months. Thus the rabbits skeleton might not fully mature in this research. Another possible reason is that during the research, the rabbits were found to gain significant weight. Based on Huiskes (2000b) theory, this increased workload will stimulate the bone growth which countered the ovariectomy effect to some extent in this research. Thus an improved version of the current research may be carried out with older rabbits and also with weight control.

The sample size in this research is relative small. The 12 bone cubes were divided by four (two treatment categories: OVX and INTACT group; two condyle categories: lateral and medial condyle) and resulted in only three cubes in each statistic group, which is actually the lower limit for statistic analysis. In addition, large variance was observed
for the OVX groups in apparent stiffness and strength analysis (Figure 4.3.5, Figure 4.3.6, Figure 4.3.9 and Figure 4.3.10). This large variance was not observed in the INTACT group which may indicate that the OVX effect was individually dependent and was not uniform from sample to sample. This called for a larger sample size to determine whether the large variance is generated randomly or caused by any potential factors. When considering increasing the sample space, the computing cost for the nonlinear models must be taken into account. This is the main issue that limited the current research in this small scale. Upgrading the hardware as well as the software may bring this research into a larger scale.

It was noticed in the current research that the machining procedure may induce error which dominates the overall errors in calibrating the trabecular bone tissue properties. The small size of the cubes made it sensitive to micro fracture. In addition, avoiding the cortical shell as well as the very porous region in the trabecular bone sometimes could not be satisfied simultaneously. The observed large difference between FEA and MTS results for some samples were possible the consequence of these difficulties.

The heterogeneous finite element models were sensitive to image quality also because the bone tissue properties were based on the image information. In this point of view, the homogeneous models are more robust. For the heterogeneous models, the images are required to be taken at same configurations particularly at the same voltage. If this requirement is not satisfied, correction must be made to compensate the error. In addition, phantom quality is also very critical for the models to be accurate. This requires the phantom to have sufficient size and to avoid any beam hardening effect.
CHAPTER 6. CONCLUSION

1. Nonlinear heterogeneous finite element models, of which the bone tissue properties are correlated to micro-CT number (Hounsfield unit), can determine the trabecular bone apparent stiffness and strength accurately. Heterogeneous modeling is more accurate than homogeneous modeling as demonstrated in this research, especially when monitoring the bone quality changes over time.

2. The \textit{in vivo} finite element models were proven to be accurate. This accuracy depends on some necessary corrections that must be made. As for this research, the bone tissue properties were calibrated using \textit{in vitro} trabecular models. Thus when applying these properties on \textit{in vivo} models, the volume fraction discrepancy between \textit{in vivo} and \textit{in vitro} models must be corrected. After these corrections, the \textit{in vivo} heterogeneous models have a high fidelity to the mechanical experiments.

3. Rigid registration applied on images with small geometry variance is accurate. This has been proven in the current research from both the validation experiment and the final application. Also, the developed rigid registration technique is not suitable when significant image variation is present.

4. The technique of combining the nonlinear heterogeneous finite element models and image registration is more sensitive to the changes of trabecular bone quality which may include bone mass, architecture and tissue material properties. This technique has great promise to evaluate and assess osteoporosis or fracture healing. The evaluation is
able to not only determine the trabecular bone mechanical property changes over time, which is owing to the accuracy of the registration tool, but also provide accurate mechanical property information at any particular point of time.

5. Homogeneous finite element models were also proven to be accurate when the material properties were carefully calibrated. But the homogeneous models were also proven to be less versatile than heterogeneous models for trabecular bone modeling when the material properties (bone tissue properties) are subjected to change.
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Appendix: Sample program

**Model name 785RLT0_2_FEM**
**Model generated at 11:36 3 16 2006**
**a=1.65E+004, b=0.23 c=1.99E-002**
**Array number (for internal use 373 )**
**
************************************************
**
*HEADING
Stiffness and yielding strength.785RLT0_2_FEM 1.65E+004, 0.23,1.99E-002
**
** Mesh definition
**
*NODE, NSET=BONE
**
**Import node definition file
*INCLUDE, INPUT=N_def.inp
**
**Define element type
*ELEMENT, TYPE=C3D8, ELSET=BONE
**
**Import element definition
*INCLUDE, INPUT=E_def.inp
**
**Import element set definition
*INCLUDE, INPUT=E_set.inp
**
** Section definition (Here only display a part of the whole set)
...
...
...
*SOLID SECTION, ELSET=Element1020, MATERIAL=Tissue1020
*SOLID SECTION, ELSET=Element1026, MATERIAL=Tissue1026
*SOLID SECTION, ELSET=Element1031, MATERIAL=Tissue1031
...
...
...
** Import the node set definition
*INCLUDE, INPUT=N_set.inp
**
**Material properties definition (Here only display a part of the whole definition)
...
...
...
*MATERIAL, NAME=Tissue1020
*ELASTIC
9.781569e+003, 0.3
*CAST IRON PLASTICITY
0.49
*CAST IRON TENSION HARDENING
97.327, 0.0
195.142, 0.2
*CAST IRON COMPRESSION HARDENING
194.653, 0.0
292.469, 0.2
**
*MATERIAL, NAME=Tissue1026
*ELASTIC
9.794773e+003, 0.3
*CAST IRON PLASTICITY
0.49
*CAST IRON TENSION HARDENING
97.458, 0.0
195.406, 0.2
*CAST IRON COMPRESSION HARDENING
194.916, 0.0
292.864, 0.2
**
*MATERIAL, NAME=Tissue1031
*ELASTIC
9.805731e+003, 0.3
*CAST IRON PLASTICITY
0.49
*CAST IRON TENSION HARDENING
97.567, 0.0
195.624, 0.2
*CAST IRON COMPRESSION HARDENING
195.134, 0.0
293.191, 0.2
...
...
...
**
**Turn on the geometry nonlinearity
**Use the asymmetric matrix to store the stiffness matrix
**Use default Newton’s solver
*STEP, NLGEOM=YES, UNSYMM=YES
*STATIC
0.2, 1.0, 1.0E-005, 0.2
**
**Boundary conditions
**
*BOUNDARY
Loadset, 3, 3,-0.0806
Boundset, 3, 3
PinSet, PINNED
FixSet, 1, 1
**
**Output requests
**
*OUTPUT, FIELD, FREQUENCY=1
*NODE OUTPUT
** require the output for the reaction force and displacement.
RF,U
**
*END STEP

For the sake of integrity, a part of the node/element definition/set files is listed below.

File name: N_def.inp (node definition)

1, 0.0000, 0.0000, 0.0000
2, 0.0560, 0.0000, 0.0000
3, 0.1120, 0.0000, 0.0000
4, 0.1680, 0.0000, 0.0000
5, 0.2240, 0.0000, 0.0000
...
...
...

File name: E_def.inp (element definition)

1, 3141, 3140, 3174, 3175, 2, 1, 32, 33
2, 3142, 3141, 3175, 3176, 3, 2, 33, 34
3, 3143, 3142, 3176, 3177, 4, 3, 34, 35
4, 3144, 3143, 3177, 3178, 5, 4, 35, 36
5, 3145, 3144, 3178, 3179, 6, 5, 36, 37
...
...
...

File name: N_set.inp (node set)

*NSET, NSET=LOADSET
247376,
247377,
247378,
...
...
...

*NSET, NSET=DISPSET
250625
*NSET, NSET=BOUNDSET
1,
2,
3,
4,
5,
...
...
...

File name: E_set.inp (element set)

*ELSET, ELSET=Element1020
143
225
378
1641
1654
...
...

*ELSET, ELSET=Element1090
106
130
133
427
...
...
...
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