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Microstructural Models
for Materials with Porous Structure

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Supervisor of the doctoral thesis: prof. Ing. Ondřej Jiroušek, PhD.

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Prague, October 2014

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Abstract:
The aim of the study is to develop and validate experimental and numerical modelling techniques for the investigation of deformation behaviour of a porous material. For this purpose, a trabecular bone as biological porous material was chosen. Development of the microstructural model with a complex inner structure consisted of three main parts: i) identification of the material model; ii) a precise description of the inner structure; iii) validation of the resulting microstructural model.

Identification of the material model was based on micro (three-point bending) and nano-mechanical (nanoindentation) testing of the single trabeculae (single trabecula is the basic building element of trabecular tissue). These tests were simulated using the finite element method and the calculated results were compared (using the least square method) to experimental obtained values. In this way the best set of the material constants for elasto-visco-plastic model with damage for single trabecula were identified.

To obtain a geometrically accurate finite element model of the trabecular microstructure and suitable experimental data for model validation the time-lapse micro-computed tomography scanning of compression test was performed. The sample was placed into a loading device and subjected to incremental compressive loading and each deformation step was observed using X-ray microradiographic imaging. Radiographic data were reconstructed and a high resolution voxel model was prepared from the tomography of the undeformed state. The identified material model from nano and micro-mechanical testing was prescribed to the voxel model and the simulation of the compression test was performed to verify its reliability to predict the deformation behaviour of bone. The calculated deformation fields were compared with the corresponding deformation fields which were determined from tomographically captured load states using the digital volume correlation method.

Keywords: digital volume correlation, finite element method, microstructural model, time-lapse X-ray microradiographic imaging, nanoindentation, trabecular bone
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Abstrakt:
Cílem doktorské práce bylo navržení a odzkoušení experimentálních a modelovacích technik sloužících k vytvoření vhodného numerického mikrostrukturálního modelu, který by popisoval deformační chování materiálů s porézní strukturou. Trabekulární kost je příkladem porézního materiálu se složitou vnitřní strukturou, který je navíc biologického charakteru. Právě trabekulární kost byla v této práci použita jako reprezentativní materiál. Návrh mikrostrukturálního modelu spočíval ve třech hlavních celcích: i) identifikaci vhodného numerického materiálového modelu; ii) vymodelování složité vnitřní struktury; iii) ověření funkčnosti vytvořeného mikrostrukturálního modelu.

Identifikace numerického materiálového modelu probíhala na základě simulací provedených experimentálních zkoušek na úrovni jednotlivých trabekul (základní prvek struktury). Konkrétně na nano-úrovni byla provedena nanoindentace zkouška, na mikro-úrovni pak tříbodový ohyb trabekuly. Simulace těchto experimentů pomocí metody konečných prvků a na základě srovnání vypočtených a naměřených charakteristik byl identifikován pokročilý elasto-visco-plastický materiálový model s poškozením na úrovni jedné trabekuly.


Klíčová slova: digitální korelace objemu, metoda konečných prvků, mikrostrukturální model, mikro-tomografie, nanoindentace, trabekulární kost
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Chapter 1

Introduction

Materials with complex inner structure are materials which are common in nature. Typical representation of these type of materials are porous (or cellular) solid materials. Their inner structure contains large amounts of pores which are typically filled with a fluid (liquid or gas). The ratio between a solid state and void spaces is called porosity and porosity ratio has significant influence on mechanical properties. From a mechanical point of view these structures have some unique properties, e.g. the ability to undergo relatively high loading despite very low specific weights. On the other hand, the foam-like structures are able to absorb a high portion of deformation energy with makes them ideal for absorbing the energy of impacts. One of the most widespread biological materials in nature is bone. Bone as a structural component of bodies consists of two principal types of bone tissues: cortical (or compact) and trabecular (or cancellous, or spongy) [1]. Cortical bone is dense bone with a lamellar (or layered) structure with a low porosity level (mostly up to 10%) and is found in long bones (femur, tibia, fibula, etc.) whereas trabecular bone is light and a highly porous (with typical porosity 75 − 95%) tissue occupies the core of long and flat bones. The porosity reduces the strength of the bone but also reduces the weight. On the other hand the trabecular bone is shaped in dependence on loading (bone remodelling process) and strength is provided only where it is needed [2]. The mechanical properties are defined by the porosity and the manner in which the inner structure is formed. Although the trabecular bone contributes to approximately 20% of the total skeletal mass (the rest is cortical bone) its presence plays an important role in the behaviour of deformation and failure of the whole bone.

The aim of this doctoral thesis is the investigation of the mechanical behaviour of a trabecular bone based on numerical models. A pure description of the architecture
of the trabecular bone as well as the material model is crucial for understanding the
deformation behaviour of the trabecular bone under loading. Currently, porous mate-
rials are attracting increasing interest in the field of regenerative medicine a branch of
translational research in tissue engineering which deals with the process of replacing
or regenerating human cells, tissues or organs to restore or establish normal function.
These biomaterials are usually employed to replace or regenerate a variety of tissue,
such as bone, cartilage etc [3]. Nowadays, artificial bone implants based on open-cell
metal foams are commercially produced (e.g. titanium metal foam PlivioPore, Synthes,
Switzerland). In terms of the trabecular bone it is essential to establish experimental
and numerical methods for the precise measurement and prediction of the behaviour
of trabecular bone and bone-like structures as well as methods for artificial bone de-
velopment and its quality assessment.

Knowledge about the deformation behaviour of bone structure is important not only for
artificial bone development but also important for investigation into the degradation
processes like osteoporosis and also into bone quality assessment. Osteoporosis is a
disease in which the density and quality of bone are reduced, leading to weakness of
the skeleton and increased risk of fracture. Osteoporosis and associated fractures are
a serious cause of mortality (up to 30% of patients die in 1 year after hip fractures
due to complications [4]) and morbidity. Osteoporosis is a global problem: 1 in 3
women (1 in 5 men) over 50 will suffer a fracture due to osteoporosis [5]. According to
the World Health Organisation (WHO), osteoporosis is second only to cardiovascular
disease as a global healthcare problem and the International Osteoporosis Foundation
(IOF) estimates that the annual direct cost of treating osteoporosis fractures of people
from the US, Canada and Europe is approximately 48 billion USD. The total cost is
predicted to rise to 131 billion USD by 2050.

1.1 Aims and objectives

One of the possibility how to investigate the mechanical behaviour of porous materials
are microstructural models. These types of models describe the complex inner structure
using basic building elements which are connected to irregular network (usually in case
of biological materials) and together create an architecture (geometry of structure).
Besides the architecture, material model for basic element of structure is needed to
determine. In this way developed numerical model including geometry and material
descriptions can be used for simulation of mechanical behaviour of structure and for
1.1. AIMS AND OBJECTIVES

prediction of its response to loading. The aim of this doctoral thesis is to develop microstructural numerical model of trabecular bone which is capable to predict the deformation behaviour of a real trabecular bone. The material model is determined at single trabecula level using two types of tests: nanoindentation of trabecular bone (nano-scale) and three-point bending test of isolated trabeculae (micro-scale). This material model is then applied to the complex sample whose architecture had been developed using micro-computed tomographic (micro-CT) imaging and simulation of compression test is performed. Finally, the microstructural model is validated using the real compression test with trabecular bone sample (macro-scale test). Special attention is placed on universality of the developed procedure in respect to using this procedure for similar porous materials (e.g. metal foams). Several sub-tasks are performed to achieve the objective:

**Single trabecula level - identification of the material model**

- single trabecula-sample preparation for the nanoindentation test
- nanoindentation test
- simulation of the nanoindentation test using Finite Element (FE) method and identification of the material model
- single trabecula-sample preparation for the three-point bending test
- performance of the three-point bending test
- the FE simulation of the three-point bending test and identification of the material model

**Development of the microstructural model**

- micro-CT imaging of the trabecular bone sample and image reconstruction
- image processing and FE model geometry development
- application of the identified material model to geometric FE model
- FE simulation of the compression test
Validation of the microstructural model

- compression test with a trabecular bone sample and micro-tomographical scanning
- calculation of strain field using Digital Volume Correlation (DVC) techniques
- comparison of experimental and FE results

1.2 Limitations

Despite the effort to preserve physiological conditions of the samples during the testing, the author is aware that it is impossible to prevent completely the degradation of testing tissue. Moreover, the samples of trabecular bone were harvested from cadaver donor and stored in formaldehyde solution. For this reasons the rate of degradation of material properties is hard to set. The second aspect is the fact that the study is aimed towards the mechanical point of view of the problem and other biological and chemical aspects are excluded.

1.3 Thesis structure

Chapter 1 introduces the topic of the doctoral thesis and defines sub-task and objective of the investigation.

Chapter 2 introduces the material (trabecular bone) which is investigated in this doctoral thesis. Chapter briefly describes the structure of the trabecular bone at different scale levels as well as a process of bone formation.

Chapter 3 deals with identification of a material model of trabecular bone based on the nanoindentation test, i.e., identification of the material model at nanol-level. The nanoindentation test and its numerical simulations to identify constants of the material models as well as a sensitivity study are described.

Chapter 4 presents material model identification based on the micromechanical three-point bending test of a single trabecula, i.e., identification of the material model at micro-level. The bending test and its numerical simulations to identify constants of
the material models are described.

**Chapter 5** focuses on the basic principle of radiographic techniques mainly as a source of data for microstructural models development. In this chapter the basic image processing algorithms and types of microstructural models are described. In-house developed tool for image processing and model development is also introduced.

**Chapter 6** describes the compression test of the trabecular bone sample using a custom loading device and micro tomographic scanning. The data measured during the test were evaluated by using DVC technique and the results were used to verify the material model (which was identified at micro and nano level).

**Chapter 7** proposes the FE model development and numerical simulation of the performed compression test. The results from the simulation are compared with the values obtained from the real compression test.

**Chapter 8** summarises the work performed in the doctoral thesis and concludes the possibilities of using a combination of modern techniques to obtain suitable microstructural model for prediction of the mechanical behaviour of porous structures.
Chapter 2

Trabecular bone

2.1 Introduction

Trabecular bone is porous material and this type of material is necessary to model at micro and macro levels. Micro level relates to basic structural elements called trabeculae whereas macro level relates to the whole architecture, i.e. irregular network of trabeculae which are interconnected. These levels are according to Ashman [6] also described in terms of material and structural properties. Structural properties are defined as the properties of both trabeculae and pores whereas material properties are defined only as inner properties of trabeculae. Structural properties play an important role in global stress analyses, while material properties related to micro level stress analyses [7].

2.2 Bone structure

Bones in human body are responsible for many different functions, e.g. structural support of body, protection of vital organs, acting tool for muscles. Besides these “acting” functions the bones also participate in reservoir function, e.g. mineral storage and physiological functions, e.g. the formation of blood vessels. The structure of the bone is astonishing because the weight is similar to light wood whereas the strength of bone is the same as cast iron (100 ÷ 200 MPa) [8].
2.2. BONE STRUCTURE

2.2.1 Hierarchical structure of bone

Bone is a composite material and its inner structure is hierarchically organised, which means that different types of entities are recognised depending on the observation level. This structure provides maximum strength with a minimum of material [3, 9]. There are two major types of bone, trabecular (or spongy) and cortical (or compact). The classification of these two types is based on the porosity and the unit microstructure. Four hierarchical levels can be distinguished for both types in adult human bone. The hierarchical levels for both types are summarised in Tab. 2.1.

Table 2.1: Hierarchical structure of bone [10]

<table>
<thead>
<tr>
<th>Level</th>
<th>Cortical bone</th>
<th>Trabecular bone</th>
<th>Size range [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Solid material</td>
<td>Solid material</td>
<td>&gt;3000</td>
</tr>
<tr>
<td>1</td>
<td>secondary osteons, primary osteons, interstitial lamellae</td>
<td>secondary trabeculae, primary trabeculae, trabecular packets</td>
<td>100÷300* 75÷200**</td>
</tr>
<tr>
<td>2</td>
<td>lamellae, lacunae, cement lines</td>
<td>lamellae, lacunae, cement lines, canaliculi</td>
<td>3÷20* 1÷20**</td>
</tr>
<tr>
<td>3</td>
<td>collagen-composite</td>
<td>collagen-composite</td>
<td>0.06÷0.6* 0.06÷0.4**</td>
</tr>
</tbody>
</table>

* cortical bone ** trabecular bone

2.2.2 Level 0 - macrostructure

Cortical bone is solid and much denser material than trabecular bone with porosity within the range of 5 ÷ 10 % [11]. Cortical bone is located primary in the shaft of long bones and forms the outer shell around the trabecular bone at the end of joints and the vertebrae. The basic first level structure of cortical bone are osteons. Unlike cortical bone trabecular bone is much more porous with porosity ranging between 50 ÷ 90 %. It is found at the end of long bones, in vertebrae and in flat bones like pelvis and provides them with supporting strength. Trabecular bone is more compliant than cortical bone and plays a significant role in distribution and dissipation of the energy from articular contact loads. Trabecular bone contributes to approximately 20 % of the total skeletal mass while cortical bone contributes to the remaining 80 % but trabecular bone has much longer surface area than cortical bone (7.0 × 10^6 mm² and 3.5 × 10^6 mm²) [10]. Bone structures with visible differences between cortical and trabecular bone are schematically depicted in Fig. 2.1 and in Fig. 2.2 differences
(mainly porosity) of these structures are captured using scanning electron microscope (SEM).

![Bone Structure Diagram](image1)

Figure 2.1: Bone structure: trabecular and cortical bone

![Bone Structure SEM Image](image2)

Figure 2.2: Bone structure captured using SEM: Difference between trabecular (right side of the image) and cortical bone (left side of the image)

### 2.2.3 Level 1 - mesostructure

On structural level 1 (and also level 2) major differences between trabecular and cortical bone structure can be found. Cortical bone is composed of osteons (also called Haversian systems) which can be found in two types: primary and secondary. In
general, these osteons differ in the forming process. Primary osteons are formed by mineralisation of cartilage, i.e. in space where bone was not present whereas secondary osteons are formed by replacement of an existing bone (remodelling process, see below). Each osteon consists of concentric bone layers, called lamellae which surround a central canal (Haversian canal). The Haversian canal contains small blood vessels responsible for the blood supply to osteocytes (individual bone cells) and bone’s nerves. Osteons are $3 \div 5$ mm long and approximately $200 \, \mu m$ in diameter and run parallel to the long axis of a bone. The boundary of the osteon is created by cement line. The space between osteons is occupied by interstitial lamellae which are the remnants of osteons that were partially absorbed during the bone remodelling process. Osteons are connected to each other (and also with Periosteum which is a layer covering the surfaces of bones) by using transverse vessels (perpendicular to the long axis of the bone) called Volkmann canals.

The basic structural entity at the first level of trabecular bone is the trabecula (see Fig. 2.3-left). Trabeculae are usually characterised as strut-like structures of typically units of mm length and thickness $200 \, \mu m$ which are organised into irregular network (see Fig. 2.3-right) where they are connected to each other. Alternatively, the struts are replaced by little plate-like structure. The structure with high irregular orientation of struts can be usually found deep in bones, away from any loaded surface, while the structure with more oriented trabeculae (parallel sheets of bone with fine struts joining them) are located just underneath loaded surface. Rarely, if the trabeculae are thick enough (approximately $300 \, \mu m$), they contain blood vessels [12]. However, trabeculae unlike osteons in general do not have a central canal with a blood vessel. Within a trabecula, trabecular packet as another structure can be found but only in secondary trabecula because it is formed during a remodelling process. Trabecular packet is an angular groupings of lamellae which are separated by cement lines of $50 \, \mu m$ thickness and $1 \, mm$ length.

### 2.2.4 Level 2 - microstructure

At this scale level two basic forms of bone are recognised which are quite a lot distinct: woven bone and lamellar bone. Woven bone rapidly grows ($4 \, \mu m$ a day) and can usually be found in all fetal bones and as for adults it is produced during fracture repair. Although the woven bone is highly mineralised, it is porous at the micron level and contains blood vessels. The woven bone is weaker and more flexible than lamellar bone and has randomly organised collagen fibers. During the aging ($2 \div 3$ years
2.2. **BONE STRUCTURE**

[Figure 2.3: Trabecular bone structure captured by using SEM: single trabecula (left); trabeculae connected to the trabecular bone (right)]

[13]), woven bone is converted into lamellar bone structure (remodelling process). The lamellar bone is more precisely organised and grows more slowly (less than 1 µm a day) than the woven bone. Collagen fibrils and their associated mineral are arranged is sheets (≈ lamellae) with 1 ÷ 5 µm thickness and mineralisation is less than in case of the woven bone. The difference between the trabecular bone and cortical bone at this level lies in the way the lamellae are arranged. In case of the trabecular bone the lamellae are longitudinally organised along the trabeculae (see Fig. 2.4) within trabecular packets, whereas in the cortical bone they are arranged concentrically. The difference is also in blood distribution because the trabecular bone does not generally contain vascular channels unlike the cortical bone. On the other hand trabecular bone has most of the same entities as the cortical bone. Both bone structures include lacunae, canaliculi, and cement lines. The lacunae are small spaces (generally 10 ÷ 20 µm) occupying interlamellar area and containing bone cells called osteocyte. The lacunae are connected with each other by microscopic canals called canaliculi (approximately 0.03 ÷ 0.22 µm diameter [11]). These narrow passageways can carry nerves, veins, or capillaries but in most cases act as conduits for bodily fluids. Cement lines create the boundary of the osteon or trabecular packet and are only found in secondary bones because they are the result of a remodelling process. The thickness of cement line is approximately 1 ÷ 5 µm.

### 2.2.5 Level 3 - nanostructure

At third level (~ molecular level) both bone tissues (trabecular and cortical) consist of the same elements, namely collagen fibril-mineral composite. The organic matrix consists primarily of the protein collagen and mineral component, i.e. crystalline salts.
Collagen comprises 90% of the matrix proteins and the rest are noncollagenous proteins. Collagen is organised in chain-like structures which are twisted into triple helices. The helices are concatenated into the fibrils and fibrils are arranged in layers [14]. The collagen fibres (occupies 10% of adult bone mass) provide the bones with ductility and tensile strength [13, 15] and ability to absorb energy [16]. The crystalline salts stored in the bone matrix (in fibrils interlayer space) are composed mainly from calcium and phosphate. Combination of these elements produces hydroxyapatite crystals (rods or plates) of hexagonal symmetry (approximately 225 nm long and 10 nm thick). Hydroxyapatite crystals fill about 65% of adult bone mass and provide the bones with the rigidity and compression strength [15]. Various diseases may be related to hydroxyapatite. The osteoporosis is connected with lack of hydroxyapatite or calcium whereas inflammation of joints and surrounding tissues (such as tendons and ligaments) is caused by settling of crystals of hydroxyapatite in joins. The rest of bone mass is water and matrix, which is formed before the mineral is deposited and creates the scaffolding for the bone. The composition of bone varies from species to species and type of bone, Cowin [13] summarised that the human bone composition is: 65% of minerals, 35% of organics matrix, cells, and water whereas Martin [15] notes the approximate bone composition for dog’s bone as: 42% of minerals, 32% of organics matrix (28% of collagen), cells, and 25% water.
2.3 Bone remodelling process

A bone is living tissue which continually adapts to its biological environment (e.g. external load, bone microdamage repair etc.) through a process called remodelling. The remodelling process begins in the fetus and becomes dominant when the bone reaches its highest level of mass (typically by early 20s). The remodelling continues throughout the whole life so the most of the adult skeleton is replaced about every 10 years [17]. Mechanical force is a crucial regulator of bone remodelling and of bone architecture in general. Mechanical load influences the process in bone not only locally (e.g. bigger bone mass in the serving arm of a professional tennis player) but also systemically as in case of a bone loss in immobilised patients or astronauts who operate in zero gravity conditions [18].

There are four types of cell in bone which are important for the remodelling process: osteoblast, osteoclast, osteocyte and bone line cell [18]. Osteoblast is a bone-forming cell which produces the organic bone matrix and supports its mineralisation. On the other hand, osteoclast is a bone degrading cell which dissolves bone minerals and enzymatically degrades extracellular proteins [19]. Osteocyte is an osteoblast-derived post-mitotic cell which occupies lacunae in interlamellar space and its main role is to maintain the correct oxygen and mineral levels in the bone (homeostasis). The fourth type of cell is bone lining cell which probably couple bone resorption to bone formation (physically defining bone remodelling compartments) [20].

Two types of remodelling processes are distinguished [21]: internal and surface remodelling. The internal remodelling refers to the mechanism in which the bulk density of the bone tissue is changed, it means the resorption (the break down of an old bone) or reinforcement of existing bone within fixed existing boundaries. The surface remodelling relates to global changes in bone geometry and reshaping of cortical surfaces. Generally the bone remodelling is a process where mature bone tissue is removed from the skeleton (bone resorption) and new bone tissue is formed (ossification). At the beginning of the remodelling process the lining cells are in a resting state on the old bone surface. After activation of the remodelling, osteoclasts gather on bone surface and create resorption pits in the bone surface which are called Howship’s lacunae. The resorption of old bone occurs using osteoclasts which remove the mineral matrix and break down the organic collagen fibres. The resorption process is finished and the osteoclasts are replaced with the osteoblast. The osteoblasts start the forming process of a new bone. The first osteoblasts create the osteoid (matrix of collagen) and then mineralise the osteoid to form a new bone. Finally, the bone returns to its resting
state. The described remodelling sequence is schematically depicted in Fig. 2.5. The remodelling process in the case of trabecular and cortical bone is the same, but with a different geometry in order to form the concentric lamellae in osteons.

![Scheme of bone remodelling process](image)

**Figure 2.5:** Scheme of bone remodelling process (taken from [22])

### 2.4 Mechanical properties of a bone

A bone is organic material and in this case it is almost impossible to determine exact value of elementary mechanical properties (elastic modulus, tensile and compressive strength etc.). Many factors, such as: age, gender, location in the body, mineral content and diseases influence these properties. For this reason the properties can be expressed in range or as an average value. In additional, the hierarchical structure of a bone and orientation of components cause that overall mechanical properties depend on the direction of loading. In general, the bone tissue is primary designed to resist load in direction of natural loading (produced by muscles, weight support, external loading during human movement etc.) while the resistance in other directions is decreased (this ability is achieved by the remodelling process which was described in section 2.3). For example, in the case of femur the preferred load is in the direction of its longitudinal axis and the strength in this direction is equivalent to the effect of 600 kg of mass while the resistance of the femur subjected to bending and torsion is significantly smaller: 300 kg and 10 kg, respectively [1].
The overall trabecular tissue mechanical properties are influenced by the same factors which lead to a different ratio of porosity, thickness of trabeculae and its arrangement and thus mechanical properties of the trabecular bone play a significant role in the behaviour of the whole bone. In many studies the ranges of measured elastic modulus using different testing methods, anatomic site and performed in various environmental conditions are published. Rho [7] and Keaveny [23] summarised that elastic moduli values of a single trabeculae are within the range $1 \div 20$ GPa and $6 \div 19$ GPa, respectively. These values were measured by using various testing methods such as ultrasonic measurement, nanoindentation, tensile testing, three and four point bending and under both environmental conditions, i.e. wet and dry. It is evident that environmental conditions as well as anatomic site have significant influence on measured moduli and a precise value has to be related to specific tissue sample. Nanoindentation technique, three-point bending test a compression test with samples of the trabecular bone as well as discussion and literature review will be described in more details in the following chapters.
Chapter 3

Identification of the material model of single trabecula based on the nanoindentation

3.1 Introduction

The beginnings of an indentation hardness test are dated back to the early last century, e.g. in 1900 J. A. Brinell invented the Brinell hardness test; in 1914 H. M. Rockwell and S. P. Rockwell co-invented and patented the Rockwell hardness tester; in 1921 the Vickers hardness test was developed by Robert L. Smith and George E. Sandland at Vickers Ltd. In the indentation test, a hard indenter with known material properties is pressed down into the sample surface and the load and penetration depth is continuously measured. The basic principle of the before mentioned types of indentation test is the same, however, the difference lies in the shape of the indenter. Brinell testing is typically performed with carbide ball indenter whereas four-sided diamond pyramid indenter is used for Vickers test. Indentation techniques were further developed during the 20\textsuperscript{th} century and with the advancement of measurement and production technology the applied loads, measured penetration depth and size of the indenter could be rapidly reduced. The advances in technology lead to the nanoindentation which was introduced in the 1970s. Unlike the Brinell test where the load applied on the indenter was in thousands of N, the peak value of the applied load during the nanoindentation is typically in units to tens of \( \mu \text{N} \). The penetration depth is then in hundreds of nanometers. The scale of indentation forces and depths enables to measure the mate-
3.2 Nanoindentation theory

Nanoindentation device is composed of three crucial components [34] which are schematically depicted in Fig. 3.1-left: indenter, actuator for applying a loading force and sensor for measuring the penetration depth. Indenter with specific geometry is mounted to a rigid column through which the force is applied from the actuator. Because of a small load the actuator is usually based on a capacitive principle. As it was mentioned before the variety of indenters made from variety of materials are used in instrumented indentation testing. Diamond is a frequently used material for nanoindentation because its high hardness and elastic modulus minimise the measured displacement caused by deformation of the indenter. Two types of indenter’s shape are mainly used: pyramidal
and spherical indenter. The most preferred pyramidal indenter is the Berkovich indenter (three-sided pyramid). This indenter has the same constant area as the Vickers indenter (four-sided pyramid) and unlike the Vicekers indenter a three-sided pyramid can be ground to point easily, thus maintaining its self-similar geometry to very small scales.

On the other hand the spherical indenter has different contact properties than a “sharp” pyramidal indenter and it is used for elimination of large gradient of contact stresses during indentation. For spherical indentation, contact stresses are initially small and produce only elastic deformation. The transition between elastic and plastic deformation occurs with a increasing load and yielding can be examined. Theoretically, a diagram corresponding to uniaxial stress-strain curve can be obtained from one spherical indentation [35].

\[
S = \frac{dP}{dh}
\]  

Figure 3.2: Nanoindentation: sketch of indentation by a tip into a specimen surface (left), nanoindentation curve with typical indentation phases (right)

Probably the most widely used methodology how to determine the elastic modulus from nanoindentation test is according to Oliver-Pharr [28]. This method is based on a slope of upper portion of the indentation curve \((S)\) which is called elastic contact stiffness (see Fig. 3.2-right) and on projected contact area \((A)\) at the load. Elastic contact stiffness can be calculated as:

\[
S = \frac{dP}{dh}
\]

where \(P\) is the applied load and \(h\) is the penetration depth. Calculation of the reduced elastic modulus \((E_r)\) is based on the elastic contact theory which was first studied by Herz [36] in the late 19\textsuperscript{th} century. The reduced elastic modulus can be determined as
3.3. NANOINDENTATION TEST OF TRABECULAR BONE

follows:

\[ E_r = \frac{\sqrt{\pi}}{2} \frac{S}{\sqrt{A}} \]  

(3.2)

where area function \( A \) for perfect Berkovich indenter is given [37]:

\[ A = 3\sqrt{3}h_c^2 \tan^2 \theta \]  

(3.3)

where \( h_c \) is contact depth, i.e. vertical distance along which the contact is made (see Fig. 3.2-left). In case of the Berkovich indenter the face angle is \( \theta = 65.27^\circ \) and Eq. 3.3 evaluates to:

\[ A = 24.494h_c^2 \approx 24.5h_c^2 \]  

(3.4)

Finally, the elastic modulus of tested material (\( E \)) is determined using:

\[ \frac{1}{E_r} = \frac{1 - \nu^2}{E} + \frac{1 - \nu_i^2}{E_i} \]  

(3.5)

where \( E_i, \nu_i \) is elastic modulus and Poisson’s ratio of the diamond tip; \( E, \nu \) is elastic modulus and Poisson’s ratio of the specimen. For diamond tip, the elastic constants \( E_i = 1141 \text{ GPa} \) and \( \nu_i = 0.07 \) are usually taken. Poisson’s ratio of the tested material has to be expertly estimated. On the other hand, in [34] it is mentioned that even roughly estimated deviation \( \pm 0.1 \) in Poisson’s ratio produces approximately 5% uncertainty in the calculation value of elastic modulus.

3.3 Nanoindentation test of trabecular bone

3.3.1 Sample preparation

Thick sample (3 mm) of the trabecular bone was cut from human (37-year male) femoral head using precision saw (Isomet 1000, Buehler GmbH, Germany). Delipidation process consisted of cleaning cycles in 1% Alconox detergent lotion (Alconox Inc., USA) in ultrasonic bath (Sonic 3, Polsonic, Poland) for 15 minutes per cycle. Between cycles the released lipids were removed from the specimen by airflow. Delipidated sample was rinsed in distilled water with temperature kept below 40°C, which is necessary to prevent the degradation of the biological samples. Reduction of roughness of the specimen surface is crucial to nanoindentation test because indentation depth is typically approximately 1 \( \mu \text{m} \). For reduction of the surface roughness to a minimum possible value the cleaned sample was embedded into a low shrinkage epoxy resin.
(EpoxyCure, Buehler GmbH, Germany) and ground using polishing machine (LaboPol-4, Struers, Denmark) with diamond grinding discs (grain sizes: 35 µm and 15 µm) and monocrystalline diamond suspensions (9 µm, 3 µm, 1 µm). Finally, aluminium-oxide suspension (Al₂O₃) with grain size 0.05 µm was used for polishing. Achieved average surface roughness was 25 nm. The sample of trabecular bone prepared for nanoindentation is shown in Fig. 3.3. The complete sample preparation procedure is described in more detail in our publication [38].

Figure 3.3: Cross-section of the human femur with femoral head which is marked by the red rectangle (left), sample of trabecular bone prepared for the nanoindentation (right)

### 3.3.2 Indentation

Embedded sample was fixed into the nanoindentation device Hysitron Triboindenter (Hysitron Inc., USA) and the nanoindentation test was performed using the Berkovich tip. Multiple indents at two positions (40 indents per position) were performed in 2 × 5 and 5 × 2 grid pattern with 35 µm grid size. Grid size was chosen with respect to the size of influenced plastic zone under the indenter which is 2 ÷ 3× larger than the maximum penetration depth. In the nanoindentation handbook it is presented that the distance between indents should be at least 5 ÷ 10× bigger than the size of a plastic zone. In the nanoindentation the maximal penetration depth was approximately 1.1 µm, and therefore the grid size was sufficient. Nanoindentation was carried out by using trapezoidal loading function with 10 mN peak force and 40 s holding times of maximum force value and four loading rates (60, 80, 120, 200 mN/min) were used. The variation of the loading rates was chosen due to more statistically significant FE fitting procedure of the nanoindentation test. The indentation control parameters are summarised in Tab. 3.1.
3.4 Simulation of the nanoindentation test

Nanoindentation is a powerful tool for the assessment of elastic material properties. On the other hand the plastic properties can not be reliably directly determined from the nanoindentation curve. Many authors assess an advanced material model based on the FE simulation of the nanoindentation test [29–33]. A simulation of the nanoindentation test is commonly designed as rotationally axisymmetric plane model due to computational simplification. In the plane model the three sided pyramidal Berkovich indenter is replaced with a cone (with internal angle 70.3°) with equivalent contact surface [32]. The sharp tip of the model of the indenter is usually rounded with a radius 100 ÷ 300 nm for better numerical convergence. Numerical model of the indenter might not be significantly affected by rounding because the manufacturers specify that the tip radius of the new Berkovich indenter is approximately 50 ÷ 150 nm and during the multiple indentations it still raises as the indenter blunts.

### Table 3.1: Control parameters of nanoindentation test

<table>
<thead>
<tr>
<th>Position</th>
<th>Pattern</th>
<th>Grid size [µm]</th>
<th>Loading rate [mN/min]</th>
<th>Holding time [s]</th>
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<tr>
<td>1</td>
<td>2 × 5</td>
<td>35</td>
<td>60</td>
<td>80</td>
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<td></td>
<td></td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200</td>
</tr>
</tbody>
</table>

3.4.1 FE model of Berkovich indenter

In this study the sharp tip of the cone which represents the Berkovich indenter in plane model was rounded with 200 nm radius according to the work published by Chen [39]. The model of the indenter was discretized using 1,063 3-nodes structural solid 2-D elements (603 nodes) with linear shape function. Mesh density was not constant, on the contrary, it is gradually increased towards the contact with the model of a specimen because at the point of the contact high distribution of stress was expected. The geometry and mesh distribution of the FE model of the indenter are in detail shown in Fig. 3.4. Linear elastic material model with known elastic modulus $E_i=1141$ GPa and Poisson’s ratio $\mu_i=0.07$ was used for FE analyses. The height of the model was set to
3.4. SIMULATION OF THE NANOINDENTATION TEST

Figure 3.4: FE model of the indenter with dimensions (left) and detailed view on the rounded tip (right) marked by the red rectangle

2 µm which is sufficient because the indentation depth was approximately 1 µm.

3.4.2 FE model of the trabecular bone

Trabecular bone was modelled using 2-D structural solid elements of a rectangular shape. It was assumed that computed displacement of the indentation tip could be influenced by inappropriate specimen dimensions which cause improper boundary conditions as well as mesh density. For the determination of suitable geometrical parameters of the model a sensitivity study was performed.

Study of geometrical parameters

Three parameters were varied during the sensitivity study: height of the sample ($h$), width of the sample ($w$) and ratio of the mesh density ($m_d$). The height and width values varied in $50 \div 300$ µm range. The varied range approximately corresponds to $50 \div 300 \times$ penetration depth. Mesh density is modified according to the value of variable $m_d$ for values: 1, 2, 4, 8, 16. The element edge length in the area of a variable zone density (see Fig. 3.5) is calculated as the product of $m_d$ and maximum element edge length in the gradient mesh distribution area ($\approx 0.66$ µm). The size of the elements in the rest areas is calculated as complementary parts to both mesh zones. Reference results for the sensitivity study were calculated for the maximum value of $h$ and $w$ in the chosen range ($h$ and $w = 300$ µm) and with the highest density of the mesh ($m_d = 1$). This model was composed of 416,728 elements and 209,350 nodes and it was assumed that this model was large enough and calculated mechanical properties were
3.4. SIMULATION OF THE NANOINDENTATION TEST

Figure 3.5: Scheme of FE model of the trabecular bone with different meshing zones (left) and detailed view on the gradient mesh distribution area (right)

not affected by the boundary conditions as well as density and quality of the mesh. On the other hand this model is too computationally expensive (computational time: 21.45 h on CPU i7 3.60 GHz) for use within the material model identification procedure which includes very large number of simulation cycles. Randomly taken experimental indentation curve was chosen from the set of measured curves and sampled to obtain force and depth values. Incremental force values were prescribed on the model of the indenter. During the sensitivity study simulations (force controlled) of each combination of $h$, $w$ and $m_d$ were taken and results compared using the least square method ($R^2$) to results of the reference model. Coefficient $R^2$ was calculated as:

$$R^2 = 1 - \frac{\text{sum of residual squares}}{\text{total sum of squares}} = 1 - \frac{\sum_{i=1}^{n}(RD_i - CD_i)^2}{\sum_{i=1}^{n}(RD_i - RD)^2} \quad (3.6)$$

where $RD_i$ represents the $i$ value of depth (on the sampled indentation curve) in the reference model, $CD_i$ represents the $i$ value of depth in the computed model and $RD$ represents the mean value of depths of the reference model. Goodness of the fit is shown as an $R^2$ value ($0 < R^2 < 1$). The value of $R^2 = 1.0$ indicates a perfect fit, whereas $R^2 = 0.0$ indicates that the model might be unsuitable for this analysis. The results from sensitivity study of geometric parameters are sorted according to their $R^2$ values and summarised in Tab. 3.2.
3.4. SIMULATION OF THE NANOINDENTATION TEST

Table 3.2: Results of sensitivity study of geometrical parameters

<table>
<thead>
<tr>
<th>order</th>
<th>(h) [(\mu m)]</th>
<th>(w) [(\mu m)]</th>
<th>(m_d) [-]</th>
<th>n. of elem.</th>
<th>reduced c.</th>
<th>(\Delta d) [nm]</th>
<th>(R^2) [-]</th>
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<tr>
<td>1</td>
<td>300</td>
<td>300</td>
<td>1</td>
<td>416,728</td>
<td>1</td>
<td>-</td>
<td>1</td>
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<tr>
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<td>13.463</td>
<td>9.959</td>
<td>0.9989327997</td>
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</table>

From the results it is obvious that size of \(h\) plays crucial role, i.e., dimension in loading direction. The width \((w)\) of the model of the sample is not essential, however, it should not be lower than the minimum value 50 \(\mu m\). Although, modification of the mesh density provides an estimated effect, i.e., the model with lower mesh density predicts indentation depth less accurate, some level of mesh reduction is necessary for simplification of the complexity of the model. Finally, the influence of the degree of the shape functions of the elements was studied. Simulations with quadratic and linear shape functions of elements were carried out and resulting indentation curves were compared. The difference between curves was negligible while computational time in the case of the quadratic elements was significantly larger. On the basis of the sensitivity study, the model with linear elements, \(h = 150 \, \mu m\), \(w = 70 \, \mu m\) and \(m_d = 8\) was chosen for the further material identification procedure. Combination of these parameters gave error \(R^2 = 0.999997022937\) and the error of the penetration depth \(\Delta d = 0.457 \, nm\) (at the end of the loading phase) comparing the reference model. These values are close enough to the results of the reference model. For this combination of geometrical parameters the computational time was significantly reduced to 0.0334 of computational time of the reference model. The conclusions from the sensitivity study are similar to the results presented by Poon [40].

Material model of the trabecular bone

Proper material model of the trabecular bone as well as the geometrical model of the nanoindentation is necessary for FE analyses. For full description of the mechanical behaviour of the trabecular bone during the nanoindentation test described using the indentation curve, the elasto-visco-plastic material model with damage is necessary.
3.4. SIMULATION OF THE NANOINDENTATION TEST

Plastic behaviour of the tested trabecular bone is obvious from the residual indentation depth after total unloading while visco properties are evident from the part of holding of the constant maximum force where the penetration depth rises. The necessity to use the damage model is based on a “rounded” shape of the indentation curve in the final part of the unloading phase. This constitutive model is defined by ten following constants:

- Young’s modulus ($E$) and Poisson’s ratio ($\nu$) that are needed for the description of elastic behaviour
- yield point ($\sigma_y$) that describes plasticity using von Mises yield criterion and tangent modulus ($E_{tan}$) for description of bilinear isotropic hardening
- four constants ($C_1, C_2, C_3, C_4$) for implicit creep with time hardening according to the equation:

$$\dot{\varepsilon}_{cr} = C_1 \sigma^2 t^3 e^{-C_4/T}$$ (3.7)

where $\dot{\varepsilon}_{cr}$ is the change in equivalent creep strain with the respect to time, $\sigma$ is the equivalent stress, $t$ is the time at the end of the sub-step and $T$ is the temperature
- constants ($D_1, D_2$) for isotropic damage model based on the rate of equivalent plastic strain accumulated in element and published by Zhang [33]:

$$E_{new} = (1 - d_c) E_0$$ (3.8)

$$d_c = D_1 (1 - e^{-D_2 \varepsilon_{eqa}^p})$$ (3.9)

where $E_{new}$ is the degraded Young’s modulus of an element which is evaluated at the end of each load-step, $E_0$ is the initial Young’s modulus of the element and $\varepsilon_{eqa}^p$ is the accumulated equivalent plastic strain in the element at the end of a load-step according to:

$$\varepsilon_{eqa}^p = \sum \Delta \varepsilon_{eq}^p$$ (3.10)

$$\Delta \varepsilon_{eq}^p = \frac{\sqrt{2}}{3} \left[ (\Delta \varepsilon_x^p - \Delta \varepsilon_y^p)^2 + (\Delta \varepsilon_y^p - \Delta \varepsilon_z^p)^2 + (\Delta \varepsilon_z^p - \Delta \varepsilon_x^p)^2 + \frac{3}{2} (\Delta \gamma_{xy}^p + \Delta \gamma_{yz}^p + \Delta \gamma_{zx}^p) \right]^{\frac{1}{2}}$$ (3.11)

where $\varepsilon_x, \varepsilon_y, \cdots, \gamma_{zx}$ are appropriate strain components. Equation 3.11 is derived
from the von Mises equivalent strain:

\[
\varepsilon_{eq} = \frac{1}{\sqrt{2(1 + \nu')}} \left[ (\varepsilon_x - \varepsilon_y)^2 + (\varepsilon_y - \varepsilon_z)^2 + (\varepsilon_z - \varepsilon_x)^2 + \frac{3}{2}(\gamma_{xy}^2 + \gamma_{yz}^2 + \gamma_{xz}^2) \right]^{\frac{1}{2}}
\]

(3.12)

where in case of plastic deformation the effective Poisson’s ratio is \(\nu' = 0.5\).

The damage model as a function of the equivalent strain depends on variables \(D_1\) and \(D_2\) and this dependency is shown in Fig. 3.6. Constant \(D_1\) determines the maximum degradation rate and constant \(D_2\) governs the rate in which degradation approaches this limiting value. It would be ineffective to apply the damage model to all elements of the model of trabecular bone. Instead, size of the damage model application zone is determined based on the equivalent plastic strain distribution caused by 10 µN peak force (see Fig. 3.7) as 5 µm rectangular area. This area is sufficiently large with regards to size of equivalent plastic strain distribution.
3.5. MATERIAL MODEL IDENTIFICATION

Smoothed measured indentation curve was sampled to obtain the force values for FE analyses. These values were prescribed on an upper line of the model of the indenter in load-steps. Bottom line of the model of the trabecular bone was fixed and between the indenter and the trabecular bone contact elements were created. Calculation was divided into load-steps and pre-conditioned conjugate gradient (PCG) iterative equation solver implemented in Ansys (Ansys Inc., USA) FE code with $10^{-8}$ solver tolerance value was used. Simulations were controlled using an optimisation procedure to identify the constants of the material model.

The custom optimisation procedure was developed for solution of the problem of identification of constitutive parameters from nanoindentation curves. The procedure consists of two main parts: initialisation loop (at which the initial ranges of the searched constants are set by a user) and an optimisation loop (at which the optimum parameters are found). Several sets of material constants are randomly generated at the beginning of the initialisation loop within expertly determined ranges. The simulation of the nanoindentation test is performed for each set of constants and the resulting indentation curve is compared to the experimental values using the least square method and the coefficient of determination $R^2$ is calculated using Equation 3.6. The highest value of $R^2$ is selected and compared to the threshold defined by the user. The threshold value is imposed to increase the computational efficiency of the algorithm and to

Figure 3.7: Equivalent plastic strain distribution caused by 10 \( \mu \)N peak force (first) and detailed view of the corresponding size of meshed area (second) depicted from the whole model (third).
3.5. MATERIAL MODEL IDENTIFICATION

Figure 3.8: Flowchart of the optimisation algorithm

eliminate possibly inaccurate initial estimates as values further entered into the optimisation loop. If the $R^2$ of the selected solution is larger than the threshold defined by the user, the optimisation loop is started. As the first step in the optimisation loop, the best three solutions from previous simulations are taken and sorted according to their $R^2$ values. The property ranges for each material parameter are determined based on maximum and minimum values of these selected solutions. Then the optimisation loop randomly generates new sets of parameters using the Gaussian distribution function. Simulations with new sets of parameters are carried out and $R^2$ errors are calculated. The optimisation loop is repeated until the maximum number of iterations defined by a user is reached. The results of the last iteration provide the best-fit of material
3.6 RESULTS AND DISCUSSIONS

Only the elastic modulus could be directly determined (by Oliver-Pharr method) from the nanoindentation test and compared to the values identified from FE analyses. The remaining constants for the selected elasto-visco-plastic material model with damage were identified by using the inverse FE analysis of the nanoindentation test. These constants were not compared with experimentally obtained value because visco-plastic properties could not be estimated in nanoindentation with Berkovich tip. The plastic and purely elastic zone are not distinguishable in the loading phase due to sharp shape of the indenter. In case of the spherical indentation it is possible to calcu-
lated plastic properties from partial unloading technique [41]. This technique consists of multiple unloading phases during the main loading phase. Each unloading cycle is evaluated for the elastic modulus and the stress-strain couple. From this data the uniaxial stress-strain diagram and yield stress and tangent modulus can be established as was demonstrated in our work [42]. Unfortunately, this technique was designed for metal material and spherical indenter. Radius of spherical indenters usually used in nanoindentation is typically 5 µm or 10 µm and in comparison with trabecular lamellae thickness (1 ÷ 5 µm) are very coarse.

For statistically significant results of the numerical analysis, the emphasis was put on variety of control parameters (peak forces, holding times, loading rates) in the set of curves which were used for simulation. The resulting constants were determined by minimising the difference (established by using the least squares approach) between the experimental load-penetration depth curves and the curves obtained from the FE simulations and constants that correspond to the best fit are summarised in Tab. 3.4. The comparison of selected experimentally obtained nanoindentation curves and the curves from the FE analyses for the best fit is plotted in Fig. 3.9.

Table 3.4: Material constants inversely determined from the nanoindentation of human trabecular bone

<table>
<thead>
<tr>
<th></th>
<th>Experiment</th>
<th>FEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$ [GPa]</td>
<td>15.39±1.4 (9.1%)</td>
<td>11.6±2.6 (22.4%)</td>
</tr>
<tr>
<td>$\nu$ [-]</td>
<td>0.2(^1)</td>
<td>0.17±0.04 (21.1%)</td>
</tr>
<tr>
<td>$\sigma_y$ [MPa]</td>
<td>-</td>
<td>180±43 (24.3%)</td>
</tr>
<tr>
<td>$E_{\text{tan}}$ [MPa]</td>
<td>-</td>
<td>1854±336 (18.1%)</td>
</tr>
<tr>
<td>$C_1$ [-]</td>
<td>-</td>
<td>(3.10 ± 4.08) × 10(^{-18}) (135%)</td>
</tr>
<tr>
<td>$C_2$ [-]</td>
<td>-</td>
<td>6.10±0.42 (6.9%)</td>
</tr>
<tr>
<td>$C_3$ [-]</td>
<td>-</td>
<td>0.88±0.71 (80.7%)</td>
</tr>
<tr>
<td>$C_4$ [-]</td>
<td>-</td>
<td>-(^2)</td>
</tr>
<tr>
<td>$D_1$ [-]</td>
<td>-</td>
<td>0.73±0.04 (5.1%)</td>
</tr>
<tr>
<td>$D_2$ [-]</td>
<td>-</td>
<td>25.3±7.5 (29.4%)</td>
</tr>
</tbody>
</table>

\(^1\) expertly determined, \(^2\) not varied

From the results it is evident that some constants have standard deviations close to or larger than 25 %, namely $\sigma_y$, $C_1$, $C_3$, $D_2$. This deviation may be attributed to several aspects, e.g.: (i) the trabecular bone is a biological material and its material properties
3.6. RESULTS AND DISCUSSIONS

Figure 3.9: Comparison of the selected resulting nanoindentation curves for the best set of constants obtained from the FE analyses with the corresponding measured indentation curves

are site-dependent. The indentation curves used in FE analyses were obtained from several indentation matrices, consequently, on various trabeculae; (ii) different material model sensitivity for identified constants. To check the second assumption a sensitivity study was performed.

3.6.1 Sensitivity study

The sensitivity study was performed for one indent and the resulting curves for each studied parameter are shown in Fig. 3.10. From the performed sensitivity study the following conclusions can be made:

- From variation of yield point value it is obvious that the sensitivity decreases with increasing $\sigma_y$ value. Values 100 MPa can be considered as a threshold value because higher values have minimum effect on the curve change. Thus the small changes of curves which are observed in range 100 ÷ 300 MPa are the reason for relatively large deviation (24.3 %) of the resulting yield stress value.

- Plastic hardening is proportional to increasing value of $E_{tan}$ within the varied range.

- Equivalent strain during creep phase of the calculated indentation curve is de-
Figure 3.10: Sensitivity study for constants of the elasto-visco-plastic material model with isotropic damage (x-axis: indentation depth [nm]; y-axis: load [mN])

scribed using four constants (see Eq. 3.7), namely: $C_1$, $C_2$, $C_3$ and $C_4$. The variation of $C_1$ indicates that values of $C_1$ should be varied in range up to order $10^{-18}$. Lower values have zero effect on the calculated indentation curves whereas higher values lead to disproportionately larger creep. After the exclusion of low
values (lower than \(10^{-18}\)) from calculation of mean values of \(C_1\), the standard deviation was decreased to \(2.06 \times 10^{-18} \pm 9.72 \times 10^{-19}\) (48%). Constant \(C_2\) shows that upper limit should be set to \(\sim 6\). The values above this limit overestimated the creep function comparing measured values. The effect of constant \(C_3\) is similar as in case of \(C_2\) and from the graph in Fig. 3.10 (row 3, column 1) it is obvious that constant \(C_3\) has significant effect only for values between 1.9\(\div\)3.5. The identified result value 0.88\(\pm\)0.71 is outside of this range and its influence for the material model is insignificant. Constant \(C_4\) relates to temperature and its insensitivity to the shape of indentation curve within varied range just confirms a zero effect of temperature during the FE analysis. For this reason \(C_4\) constant can be identified during FE analyses.

- The damage model as a function of the equivalent strain depends on variables \(D_1\) and \(D_2\) and this dependency is shown in Fig. 3.6. The influence of these constants on the nanoindentation curve is predictable and shown in Fig. 3.10 (row 4, column 1 and 2). For increasing values of \(D_1\) and \(D_2\) the increased penetration depth for the same load value is obtained.
Chapter 4

Identification of the material model of the single trabecula based on the three-point bending test

4.1 Introduction

In the previous section, nanoindentation has been described as promising technique to investigate mechanical properties at nano-scale. However, this method can be reliably used only to get information about elastic properties. Yield properties as well as softening behaviour cannot be reliably measured by nanoindentation and have to be obtained by fitting to experimental data. The measured elastic properties obtained from nanoindentation are very localised and overall mechanical properties of an inhomogeneous sample can be estimated on the basis of averaging of the values from multiple indentation locations. Other disadvantages are a relatively demanding sample preparation and time-consuming indentation process. In contrast, micro-mechanical testing enable to study overall elastic as well as plastic material properties (yield stress and strain) of samples at micro-scale. With regard to dimensions of trabecular samples this scale is suitable for measurement of its overall mechanical properties. The compression/tension test and three-point bending test are essential mechanical testing methods and can be successfully used at macro-scale [43–46]. For these tests the crucial requirement is to keep proper boundary conditions during the test. In compression/tensile test a specimen is commonly fixed to holders using glue to avoid slippage. Since the sample should be subjected only to uniaxial loading, fixing procedure can be challenging in
case of an irregular shape of the sample. On the other hand, the three-point bending test does not require fixation of the sample but it is sensitive to a setup arrangement. According to the three-point bending theory a loading should be applied on the sample in the middle between supports. The advantage of micromechanical testing is the fact that stress-strain diagram can be relatively simply determined from the measured data. Applied force is usually measured directly using an embedded load-cell while the deformation of the sample can be obtained in different approaches. The basic approach is to evaluate strain of the sample from movement of loading grips or elongation measurement using laser interferometer [45]. Advanced methods as optical [43] or radiographic [47] data evaluation using Digital Image correlation (DIC) or Digital Volume Correlation (DVC) techniques are used for precise description of the strain fields on/in sample surface/volume. These techniques are useful as for the complex structure of samples and their inhomogeneous deformation. From measured force, sample dimensions, setup arrangement and strain it is possible to obtain the stress-strain diagram. Assessment of elasto-plastic properties from stress-strain diagram depends on the type of material.

4.2 Theory of three-point bending

Generally, bending theory characterises the behaviour of a slim structural element which is perpendicularly loaded to its longitudinal axis. The structural element can be called beam when its length is considerably larger than its width and thickness. Normal and shear stresses in cross-section of beam depend on the magnitude of shear force and bending moment. If the beam is only subjected to bending moment (shear force is zero) a simple bending theory can be used. In the case of a sufficiently slender beam (for practical purposes the ratio of their length to the cross-sectional dimensions is over about 10:1 [48]) deformation and stress values can be calculated by using the Bernoulli-Navier hypothesis. The major assumption of the hypothesis is that cross-sections remain planar after loading and perpendicular to the deflected centroidal axis of the beam. The deformations due to shear across the section are negligible. It is assumed that sections \( mm \) and \( pp \) (see Fig. 4.1-bottom) are mutually rotated around the axis perpendicular to the plane of bending.

Fibres in the outer surface of the beam (see Fig. 4.1-bottom) subjected to bending moment are in a state of tension, while fibres in inner surface are in compression. Between these fibres the neutral plane is found in which there are no longitudinal stresses or strains. In longitudinal cross-section of the beam the neutral plane is represented
4.2. THEORY OF THREE-POINT BENDING

Figure 4.1: Scheme of three-point bending (left-top); normal stress distribution (right-top) and scheme of rotation of the sections during the bending (bottom).

Simple bending is an uniaxial stress state where the normal stress $\sigma_x(y)$ is distributed over the cross section linearly. Stress calculation is based on deformation of the fibre $ss'$ shown in Fig. 4.1-bottom, similarity of triangles ($non_1$ a $s_1os'$) and Hooke’s law:

$$\sigma_x = E \cdot \varepsilon_x = E \cdot \frac{s's_1}{nn_1} = E \cdot \frac{y}{r}$$  \hspace{1cm} (4.1)

From the moment equilibrium equation around the $z$ axis:

$$\int \sigma_x y \, dA = \int \frac{E}{r} y^2 \, dA = \frac{E}{r} I_z = M$$  \hspace{1cm} (4.2)

where $M$ is moment of the inner forces, $I_z$ is moment of inertia to neutral axis. Substitution from equation 4.1 may be $\sigma_x(y)$ expressed as:

$$\sigma_x = \frac{M}{I_z} y$$  \hspace{1cm} (4.3)

The three point bending test is an essential experiment in mechanics. It represents the case of a beam resting on two roller supports and loaded in the middle. The shear
force is constant ($\pm P/2$) and it changes sign in the middle of the beam (see Fig 4.2).

Figure 4.2: Diagram of the bending moment ($M_y$) and shear force ($T$) for three-point bending.

The bending moment varies linearly from one end and in the midspan it reaches its maximum value ($P \cdot l/4$). The calculation of the deformation of the beam during the three-point bending is based on differential equation of the bending curve:

$$EI_x \frac{d^2w}{dx^2} = -M_{y(x)} \quad (4.4)$$

Integration of Eq. 4.4 with known bending moment functions $M_{y(x)}$ and boundary conditions (slope of the bending curve in the centre, deflection at ends) the deflection of the beam in the place of the applied load ($l/2$) can be calculated:

$$w(\frac{l}{2}) = \frac{P l^3}{48 E I_x} \Rightarrow E = \frac{P l^3}{48 I_x w(\frac{l}{2})} \quad (4.5)$$

Substitution of $E$ from Eq. 4.5 and $\sigma$ from Eq. 4.3 to Hooke’s law the deformation during the three-point banding can be expressed:

$$\varepsilon = \frac{\sigma}{E} = \frac{M}{E I_x y} = \frac{P l^3}{48 E I_x w(\frac{l}{2})} = \frac{12 w(\frac{l}{2})}{l^2} \quad (4.6)$$

In equation 4.5 it is assumed that the deflection is caused only by the bending moment, however, a normally loaded beam is subject to both bending and shear forces. Additional deflection due to shear stress can be calculated from integration of shear strain and in the case of the three-point bending is expressed in middle of the beam.
as:

\[ w_{\text{shear}}^{(\frac{l}{2})} = \frac{Pl}{4GA^*} = \frac{Pl(1 + \mu)}{2EA^*} \]  

(4.7)

where \( A^* \) is the reduced beam cross-sectional area and \( \mu \) is Poisson’s ratio. The total deflection in middle of the beam is given by:

\[ w^{(\frac{l}{2})} = w^{(\frac{l}{2})} + w_{\text{shear}}^{(\frac{l}{2})} = \frac{Pl^3}{48EI_z} + \frac{Pl(1 + \mu)}{2EA^*} \]  

(4.8)

The influence of shear force for deflection of the slender beam with ratio 10:1 (length:cross-sectional dimensions) with rectangular cross-section is approximately 4 % [49]. Thus, the decision whether to include the effect of shear in calculation of deflection depends mainly on geometrical parameters of the beam. For all tested single trabecula samples the length:cross-sectional ratio satisfied the requirement of slender beam and effect of the shear force could not be considered.

4.3 Three-point bending test of the single trabecula

4.3.1 Sample preparation

Samples of trabeculae were extracted under a magnifying glass (4 × magnification) from the proximal human femur of the same donor as in the case of the nanoindentation using a sharp-tip scalpel and pair of tweezers. Trabeculae were cleaned off marrow and grease in a detergent lotion in an ultrasonic bath. Finally, trabeculae were soaked in toner lotion to achieve an artificial particle pattern with sufficient contrast on the sample surfaces. The contrast of the surface is crucial for practicability and accuracy of evaluation of deflection of the sample using DIC method.

4.3.2 Three-point bending test

The three-point bending test was performed using a custom experimental setup which is depicted in Fig. 4.3. This setup was designed with respect to its high modularity and ability to easy modification or improvement. The experimental setup was composed of translational stages (Standa Ltd., Lithuania) which enable to adapt the setup (length between supports etc.) to dimensions of the sample. The loading was
controlled by a stepper motor (SX16, Microcon, Czech Republic) attached to a gear unit (P42, Transtecco Ltd., Italy) and the force was applied by using a precision linear stage (M-UMR3.5, Newport Corp., USA) with differential micrometer (DM11-5, Newport Corp., USA) of 0.1 µm sensitivity and 5 mm travel range. The experimental setup can be equipped with various load cells to achieve suitable measuring range and accuracy. The three-point bending tests of trabeculae were performed by using 2.2 N load sensor (FBB350, FUTEK Advanced Sensor Technology Inc., USA). For optical measurement of deflection of the sample the experimental setup was equipped with a CCD camera (Manta 504-G, Allied Vision Technologies GmbH, Germany) with 2452 × 2056 px resolution and 9 fps maximum frame rate attached to an optical microscope (Navitar Inc., USA). The working area was illuminated by a halogen light source. The experimental procedure was controlled by LinuxCNC application and custom scripts for image and force recording.

The tracking of deflection of the sample was performed using DIC toolkit [50] based on Lucas-Kanade algorithm [51] implemented in Matlab. The concept of using correlation to measure movement in digital images was first used in 1975. The basic principle of DIC is tracking of the same points (or pixels) between two images (reference and deformed subset) recorded during the deformation process. The similarity degree between these two deformation states is evaluated based on a cross-correlation (CC) or
4.3. THREE-POINT BENDING TEST OF THE SINGLE TRABECULA

sum-squared difference (SSD) criterion (the criteria are summarised in more detail in [52]). The matching procedure consists of searching the maximum value of a correlation coefficient that is determined by pixel intensity in reference and deformed image. Once the peak value of the correlation coefficient is detected, the position of the subset in deformed image is obtained. Therefore the coordinates of the points in reference subset can be mapped to points in deformed subset according to shape function (commonly the first order or second order shape function) also called the displacement mapping function. The calculated coordinates of points in the deformed subset can be located between pixels (i.e. sub-pixel locations). For this reason the intensity of the points with sub-pixel accuracy must be provided with sub-pixel interpolation scheme before the correlation coefficient calculation is made. Many sub-pixel interpolation schemes are presented in literature (bilinear interpolation, bicubic interpolation, bicubic B-spline interpolation etc.), however, in [53] the high-order interpolation scheme such as the bicubic spline interpolation scheme is highly recommended because it provides better accuracy and convergence of algorithm.

Before the testing of trabecular specimens the precision of the experimental setup was evaluated by testing of two types of specimen of known material properties, namely BoPET film (Biaxially-oriented polyethylene terephthalate, DuPont, USA) and Co-Ni wire. The overall precision of measurement was determined as the upper limit of all measurements. Measurement errors $\epsilon_E=5\%$ and $\epsilon_{\sigma_y}=7\%$ were assessed for elastic modulus and yield stress, respectively. More details on evaluation of the measurement precision can be found in our publication [44].

After the evaluation of the experimental setup accuracy the three-point bending tests of trabecular samples were performed and images of deflection of the sample and force log were acquired for each test. The groups of markers were selected on trabecula surface in the reference image frame and their positions were tracked in deformation states using DIC (see Fig. 4.4). During the DIC tracking it is possible that the calculation of correlation for some of the markers is unconverged (this can be caused by a significant change in brightness of a pixel in the image due to reflections, blur, large movement, etc.). The markers with lost correlation are automatically identified and eliminated from further strain calculation. The example of the loss of correlation for one marker is shown in Fig. 4.4-bottom left and marked by a green circle. The identified vertical displacements of the middle span markers (6, 7, 8) calculated using DIC in each deformation state (in each captured frame) were used for strain calculation.
4.4. NUMERICAL SIMULATION OF THE TREE-POINT BENDING TEST

Figure 4.4: DIC of three-point bending test of the trabecular bone specimen: selected markers on trabecula surface in reference frame and during maximal deflection (left); Dependency of vertical position of the markers on applied force (right)

according to Eq. 4.6 with respect to the setup and the sample dimensions:

\[ \varepsilon_t = \frac{6u_h}{l^2} \]  \hspace{1cm} (4.9)

where \( u \) is the average deflection of the mid-span markers, \( h \) is the trabecula height in the place of the applied load and \( l \) is the distance between the supports. Moment of inertia of the sample in mid-span and measured force were used for stress evaluation. The elastic modulus was established as a slope of the linear part of a stress-strain curve. As the yield point could not be easily defined on the basis on the shape of the stress-strain curve, the yield stress was determined from the same diagram by the 0.2 % offset method [54, 55].

4.4 Numerical simulation of the three-point bending test

4.4.1 Geometrical model development

The accuracy of results of the FE analysis is significantly dependent on accuracy of description of geometry of investigated sample. To achieve a good representation of the
geometry, each trabecular sample was mounted on a rotational stage and 360 projections (with 1° step size) were captured using the same CCD camera (see scheme on Fig. 4.5) which was used for observation of a sample deflection during the tree-point bending test. Acquired projections were processed using the shape-from-silhouette method

Figure 4.5: Scheme of a light tomography setup for model development using the shape-from-silhouette method

[56] based on the inverse Radon transform to obtain reconstructed cross-sections of the sample. The image segmentation process and surface generation based on marching cubes algorithm [57] were performed on reconstructed slices using an in-house segmentation and modelling software (see section 5.7). From smooth surface the tetrahedral mesh was subsequently created with Netgen software (Johannes Kepler University Linz, Linz, Austria) and exported to a general-purpose FE toolkit Ansys (v12.1, Ansys Inc., USA). Significant steps in model development process are shown in Fig. 4.6.

Figure 4.6: Development of the FE model of the single trabecula based on the shape-from-silhouette method: acquired projections from the light tomography (three selected angles); generated surface model (orange); meshed volumetric model (green)
4.4.2 Identification of material model

The FE model of the trabecula consists of 3-D 10-node tetrahedral elements with quadratic shape functions (an example of one of the FE model is shown on Fig. 4.7). Although the second-order elements are computationally more expensive than linear elements, their use is recommended for better accuracy mainly in bending analyses. Nodes corresponding to positions of correlation markers in image data were selected on the surface of the model and the same boundary conditions as in the experiment were prescribed. To simulate the bending test, the same material model (see section 3.4.2) for a single trabecula as in the case of the nanoindentation was used. Material constants were varied using the modified optimisation algorithm (described in section 3.5) where the comparison criterion was changed to a comparison of resulting displacements of selected nodes with displacements of the markers measured during the experiment.

![Figure 4.7: FE model of the single trabecula with prescribed boundary conditions which was used during the analysis](image)

4.5 Results and Discussions

Force and deflection of the single trabecula were measured during the three-point bending test. The elastic modulus and yield stress were directly determined from the stress-strain diagram. The experimental results, namely displacements of correlation markers and the applied force were used as control parameters for FE analyses with the goal to identify constants for the considered material model. Resulting constants determined from real tests and constants calculated using FE simulations are summarised in Tab. 4.1. Identification of material constants was based on the comparison of displacements of markers obtained from the experiment and simulation (see Fig. 4.8-left). Distribution of vertical displacements over the sample calculated in the analysis is depicted in Fig. 4.8-right.

Experimentally measured and numerically calculated mean values of elastic modulus of a single trabecula were $9.34 \pm 1.36$ GPa and $12.10 \pm 0.72$ GPa, respectively. The
4.5. RESULTS AND DISCUSSIONS

Table 4.1: Resulting constants of the three-point bending test of a trabecular bone experiment FEM

<table>
<thead>
<tr>
<th></th>
<th>experiment</th>
<th>FEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$ [GPa]</td>
<td>$9.34\pm1.36$ (14.5%)</td>
<td>$12.10\pm0.72$ (6%)</td>
</tr>
<tr>
<td>$\nu$ [-]</td>
<td>$-0.2\pm0.05$ (24%)</td>
<td>$0.2\pm0.05$ (24%)</td>
</tr>
<tr>
<td>$\sigma_y$ [MPa]</td>
<td>$185.6\pm42.0$ (22.6%)</td>
<td>$170.0\pm4.9$ (2.9%)</td>
</tr>
<tr>
<td>$E_{tan}$ [MPa]</td>
<td>$-1924\pm263$ (13%)</td>
<td>$1924\pm263$ (13%)</td>
</tr>
<tr>
<td>$C_1$ [-]</td>
<td>$(8.6\pm5.1)\times10^{-18}$ (59.4%)</td>
<td>$(8.6\pm5.1)\times10^{-18}$ (59.4%)</td>
</tr>
<tr>
<td>$C_2$ [-]</td>
<td>$4.7\pm0.88$ (18.6%)</td>
<td>$4.7\pm0.88$ (18.6%)</td>
</tr>
<tr>
<td>$C_3$ [-]</td>
<td>$2.1\pm0.67$ (32.7%)</td>
<td>$2.1\pm0.67$ (32.7%)</td>
</tr>
<tr>
<td>$C_4$ [-]</td>
<td>$-1$</td>
<td>$-1$</td>
</tr>
<tr>
<td>$D_1$ [-]</td>
<td>$0.52\pm0.08$ (16%)</td>
<td>$0.52\pm0.08$ (16%)</td>
</tr>
<tr>
<td>$D_2$ [-]</td>
<td>$30.1\pm1.4$ (4.6%)</td>
<td>$30.1\pm1.4$ (4.6%)</td>
</tr>
</tbody>
</table>

$^{1}$ not varied

Figure 4.8: Results of simulation of three-point bending test: comparison between calculated displacements of selected markers directly from experimental data using DIC and calculated values using inverse FE simulation (left); distribution of vertical displacements in state of the maximal deflection of the sample

discrepancy between results could be due to several aspects. For the calculation of stress and strain values from the experimental data the several simplifying assumptions were adopted (zero shear forces, a prismatic beam, sample without hidden defects etc.). It is also challenging to ensure proper boundary conditions due to the irregular shape of a trabecula. A combination of these factors can cause measurement error. On the other hand, FE analyses can also be a source of non-negligible error. Model development process simplifies the real geometry of the sample due to smoothing of the sample
surface to achieve a suitable number of elements for the computation and its shape ratio. Another error can be caused by improper boundary conditions during analyses (position of the sample relative to the real setup geometry, fixed contact between supports and the sample etc). Thus, the 29% difference between experimentally and numerically evaluated elastic modulus can be a combination of these measuring and numerical errors.

In section 3.6, results from a nanoindentation test has been described and mean value of elastic modulus $15.39 \pm 1.40$ GPa were obtained. The different mean values between the nanoindentation and the three-point bending test can be explained by the nature of experiments; a nanoindentation measurement of elastic properties is very localised and site-dependent. Usual procedure is to indent a polished sample not necessarily taken perpendicularly to the longitudinal axis of a trabecula and thus measured elastic constants can be significantly different. It was shown by Brennan [58] that elastic modulus measured by nanoindentation can vary in the cross-section of a trabecula in 5 GPa range. In the nanoindentation test the uniform distribution of indents over the cross-section of the ground trabecula was not preserved and it is possible that a predominant number of indents were obtained from the ’core’ position where the elastic modulus is the highest. On the other hand, the elastic modulus measured by three-point bending is expected to be close to the average value measured by nanoindentation in the cross-section. The second aspect can be caused by not exactly identical harvesting place of samples. The sample for the nanoindentation was prepared from the thick slice whereas isolated trabeculae were harvested directly from the cross-section of human femur. Therefore, the distance between the harvesting places of the nanoindentation sample and three-point bending samples was in the order of millimetres and the mechanical properties of the samples can be slightly different due to the bone remodelling process.

Yield point of the trabecular bone determined based on both tests were in $170 \div 185$ MPa range. Standard deviation of yield stress was relatively large ($SD_{nano} = 43$ MPa, $SD_{3pb} = 42$ MPa) and shows that plastic properties of the trabecular bones vary and yield point of the each trabecula also depend on place where the trabecula is located in the bone. In Carreta [55] yield stress of trabecular bone with relatively large value of standard deviation (approximately 25 MPa) is also presented. Although this study focuses on the bovine trabecular bone, it can be assumed that similar plastic behaviour is also valid in the case of the human bone.

A comparison of material constants inversely determined from the nanoindentation test
4.5. RESULTS AND DISCUSSIONS

and the three-point bending test is summarised in Tab. 4.2. The comparison shows similar values of elastic and plastic constants while constants related to creep properties are relatively different. This leads to an assumption that elastic and plastic properties have a significantly larger effect on trabecular bone behaviour than creep constants.

For confirmation of this assumption the simulation of the bending test was performed with resulting values of constants $C_1$, $C_2$, $C_3$ of the simulation of the nanoindentation test. The change in resulting deflection was negligible (approximately 1%).
Chapter 5

Microstructural models

5.1 Introduction

Currently, a computer power is increasing while its cost is still decreasing, numerical methods play a more important role in the investigation of mechanical properties of samples, especially for materials with a porous inner structure (tissues, foams, etc.). These materials are usually modelled as multiscale models, this means with varying levels of detail at different scales. In case of the trabecular bone, two modelling levels were chosen: level of isolated trabeculae (level of basic elements) and a structural level where the trabeculae are interconnected to a network. Approach to the identification of the material model of the single trabecula based on nano and micro level testing have been published in previous chapters 3 and 4. The material model identified at a trabecula level can be used for evaluation of material properties of the micro-structural model of the entire tissue network. To develop the entire micro-structural model for FE simulations, a complex inner structure of the sample must be reproduced. Nowadays, a powerful technique for capturing of the inner structure of the samples is the X-ray computed tomography (CT) which enables to generate a spatial dataset of the inner structure of an object from a large series of two-dimensional radiographic images (projections) taken around a single axis of rotation. Medical tomography devices are the most common type of CT scanners. These commercial devices are typically constructed and optimised (hardware and software) to perform a specific task (scanning of the patient body or body part) and the role of a human operator is limited. Thanks to optimisation, these specific tasks are performed quickly in sufficient quality and to serve its purpose perfectly (e.g. determination of diagnosis in hospital).
On the other hand, custom CT devices allow various measuring setup arrangements and hardware configurations. Their modular design typically enables the X-ray source and detector to change as well as setup geometry (distance between scanned object and detector/source) and measurement parameters (acquisition time, number of projections, current and voltage on X-ray source etc.). The role of a human operator is crucial because acquisition and data processing can be significantly influenced by the operator’s interventions. However, this custom CT devices can generate errors in the process of model development because of its 'open' design (high rate of the variability and operator’s interventions), for investigation of the samples at a scientific level are necessary.

In this study custom micro-tomography (micro-CT) device was used to obtain a pure description of a complex inner structure of a trabecular tissue, however, tomographic scanning of a sample is only the first step in a micro-structural FE model development. Each projections must be reconstructed and a set of correction filters must be applied to obtain three-dimensional image data. Resulting image data are further post-processed to achieve a meshed volumetric model which is suitable for numerical analyses. Process of the numerical model development is schematically depicted in Fig. 5.1.

![Scheme of the numerical model development process with significant functional steps](image)

**Figure 5.1:** Scheme of the numerical model development process with significant functional steps

### 5.2 Computed tomography (CT)

Computed tomography (CT) is a radiographic method which enables to create spatial image data of specific areas of the scanned object using X-rays. Basic principle is the same as in the case of radiography but during tomography a set of radiographic pro-
5.2. COMPUTED TOMOGRAPHY (CT)

jections of the sample from various angles is acquired. These projections are computed and post-processed to obtain three-dimensional image data of the object.

Radiography started in 1895 with the discovery of X-rays by the German physics professor Wilhelm Conrad Röntgen. Radiography is based on the principle that a part of X-ray is absorbed and scattered as it passes through an object (X-ray attenuation theory). Attenuation of X-rays is dependent on physical properties of the object (material, dimensions, structure etc.). When the X-ray beam travels through an object a part of radiation is absorbed (see scheme in Fig. 5.2). Amount of the absorbed intensity is proportional to the path in the object. Amount of X-ray being absorbed or scattered out of the beam in thickness \( dx \) can be expressed as follows:

\[
\frac{-dI}{I_z} = \frac{\sigma CA}{A} \, dx \quad (5.1)
\]

where \( dI \) is the change in intensity across \( dx \), \( I_z \) is the intensity entering the infinitesimal slab at \( x \), cross-section \( \sigma \) related with the rate that X-ray is removed from the incident beam, \( A \) is an area of the slab and \( C \) expresses concentration of molecules (i.e. number of molecules in \( \text{cm}^3 \)). Expression on the right side of Eq. 5.1 denotes a fraction of absorbed photons. Integration of both sides of Eq. 5.1:

\[
\int_{I_0}^{I} \frac{dI}{I_z} = \int_{0}^{a} \frac{\sigma \cdot C \cdot A}{A} \, dx \quad (5.2)
\]

\[
\ln I - \ln I_0 = \ln \left( \frac{I}{I_0} \right) = -\sigma \cdot C \cdot a \quad (5.3)
\]

\[
I = I_0 \cdot e^{-\sigma C a} = I_0 \cdot e^{-\mu a} \quad (5.4)
\]

where \( I \) is intensity of X-ray leaving the object, \( I_0 \) is intensity of X-ray entering the object at \( a = 0 \) and coefficient \( \mu = \sigma \cdot C \) is a linear attenuation coefficient gives Eq. 5.4 which is known as the Beer-Lambert law.

Figure 5.2: Scheme for derivation of the Beer-Lambert law
5.2. COMPUTED TOMOGRAPHY (CT)

During the irradiation process the X-ray beam travelling through different absorber layers is attenuated a different with degree depending on the absorption coefficient (if scattering is ignored, the attenuation coefficient equals to the absorption coefficient) of the irradiated sample volume. The X-ray beam then propagates through the object and is detected on a plane (detector) which is perpendicular to the direction of the propagation. The detector captures information about the absorption parameters of the projections. These measured values of the attenuation coefficient are commonly recalculated in the medical CT practise into Haunsfield units (HU) using Haunsfield scaled invented by Sir Godfrey N. Hounsfield who developed the first clinically useful CT machine. HU is a standardised and accepted unit for reporting and displaying reconstructed X-ray computed tomography CT values. The system of units represents an affine transformation from the measured linear attenuation coefficient into one where water is assigned a value of zero and air is assigned a value of $-1,000$. Measured linear attenuation coefficient $\mu_X$ is scaled to the corresponding HU value according to:

$$HU = 1000 \times \frac{\mu_X - \mu_{\text{water}}}{\mu_{\text{water}}}$$

(5.5)

In radiographic scanning the object is placed between a X-ray source (where X-rays are emitted) and detector (where the incident photons are detected) and a radiogram is acquired. There are two basic principle in the design of CT machines. In the medical CTs the object (patient) is placed into the device and its position is fixed during the irradiation process. Multiple projections are acquired using rotation of the X-source and the detector around the object. Movement of the X-source and the detector is synchronised to achieve its in-line position. This principle is depicted in scheme in Fig. 5.3-left. On the other hand in custom "laboratory" CTs the opposite approach is used to obtain multiple projections of the object. The object (specimen) is placed on a rotational stage while the X-source and detector are fixed (see scheme in Fig. 5.3-right). This approach enables a simpler design and smaller dimension of the device. Rotational stage is one of the reasons why this type of CTs are not used in medical practice because a living object must be during the irradiation process in constant position.
Figure 5.3: Scheme of the principle of the CTs design: X-source and detector are rotated around the object (left); the object is rotated while X-source and detector are fixed (right)

5.3 Correction filters

Before the reconstructions of measured projections a set of corrections should be applied to the radiograms to improve the quality of acquired data. The noise in the data is caused by imperfections of the setup (active and passive parts) and nature of the experiment (beam divergence, non-linear nature of attenuation etc.).

5.3.1 Dark current correction

Dark current is a phenomenon that relates to electrons accumulate in each pixel of X-ray imaging sensors (independent on X-ray source) due to thermal action. This rate depends on temperature of the sensor and on the impurities in each pixel and is proportional to the exposure time. The dark current is usually very stable and can be corrected by a series of exposures (dark field frames) with absence of any X-ray. The dark current and thermal noise can be significantly reduced by lowering the temperature of the detector using a cooling system.

5.3.2 Flat field correction

The objective is to eliminate artifacts from projections that are caused by variations (non-uniformities) in the pixel-to-pixel sensitivity of the X-ray imaging sensors. These imperfections are caused by a defect of the detector (age, manufacturing defects, phys-
5.3. CORRECTION FILTERS

ical damage, dust etc.). Flat-field frames are obtained by irradiation of the detector without a specimen. During the flat field recording it is assumed that the effect of the dark current is negligible. Correction of projections (raw acquired data) is given:

\[ I_C = \frac{I_i - I_D}{I_F - I_D} \] (5.6)

Where \( I_C \) is a corrected projection, \( I_i \) is an original projection to be corrected, \( I_D \) is a dark field frame (with non X-ray exposure) and \( I_F \) is a flat field frame (with X-ray exposure without an object).

5.3.3 Beam hardening correction

Standard X-ray tube spectra are polychromatic and radiography is burdened by the fact that attenuation process is energy dependent because harder components of the spectrum are attenuated less than softer components. This phenomenon is known as the beam hardening (BH) effect. If attenuation of the investigated object is not homogeneous (e.g. thickness of the object is varied) then the transmitted beam spectrum differs from point to point, i.e., behind thicker parts of the sample the spectrum is harder than behind thinner parts [59]. Thus the individual pixels of the X-ray detector are irradiated by a different spectrum. BH has several degradation consequences on measured radiograms: radiograms look noisy especially when low-energy photons are detected, empty holes look filled and the observed object looks flatter than it should be [60].

This effect can be suppressed or corrected in several approaches. The monochromatisation of the polychromatic X-ray source approach is time-consuming and requires a powerful X-ray source. The other way is to use linearised signal to an equivalent thickness method [59]. This method is based on Eq. 5.4 for attenuation of a monoenergetic X-ray photon flux through a homogeneous material which is extended for the full spectrum:

\[ I = \sum_{i=1}^{N} I_0 \cdot e^{-\mu(E_i)\Delta E_i} \] (5.7)

where the X-ray polychromatic spectrum is approximated by \( n \) monochromatic channels with the width of \( \Delta E_i \) and \( \mu(E_i) \) is the attenuation coefficient corresponding to the mean energy \( E_i \) of one energy channel.

The method consists of a calibration and correction step. The calibration signal \( c \) is
measured for each detector pixel using a set of filters with various thickness \( (t_1, \ldots, t_n) \) to obtain the filter-thickness dependence. The measured nonlinear relationship between calibrators thickness and counts of the photons is fitted by an exponential function in each pixel. Before the fitting the signal \( c \) is logarithmed which leads to a linear equation (instead of nonlinear). The tangents of fitting lines can be calculated as follows:

\[
a_n = \frac{t_n - t_{n+1}}{\ln(c_n) - \ln(c_{n+1})}
\]

(5.8)

During the correction the signal of the projection \( s \) is transposed into the equivalent thickness \( t_{eqv} \) for each detector pixel using the calibration data and tangents:

\[
t_{eqv} = a_n [\ln(s) - \ln(c_n)] + t_n; \quad c_n < s < c_{n+1}
\]

(5.9)

According this procedure the projections are linearised, it means that a linear dependence between the object thickness and projections transposed into equivalent thicknesses is obtained. Calibration with calibrators with similar attenuation coefficients as the inspected sample improves the correction accuracy. In Fig. 5.4 is illustrated a revolver for the BH correction where the aluminium calibrators with different thickness are placed in an individual position and can be automatically changed to speed up the calibration procedure.

![Figure 5.4: Revolver for the BH correction with attached aluminium calibrators](image)

**5.4 Tomographic reconstruction**

The goal of the tomographic reconstruction is to obtain the inner structure of a three-dimensional object from a set of its projections. For this purpose a variety of practical
reconstruction algorithms have been developed to implement the process of reconstruction. These algorithms are largely based on the Radon transform. The Radon transform and its inverse form provide the mathematical basis for reconstructing tomographic images from measured projections.

5.4.1 Radon transformation

The Radon transformation (RT) and formula for the inverse transform are mathematical techniques which were introduced in 1917 by the Austrian mathematician Johann Radon. From the mathematical point of view the attenuation of X-rays as they propagate through an object can be modelled using a line integral. The object is modelled as a two-dimensional (or three-dimensional) distribution of the attenuation while the line integral describes the total X-ray beam attenuated which is caused by travelling through the object in a straight line. The Beer-Lambert law (see Eq. 5.4) can be expressed as the line integral:

\[ p(t, \theta) = \ln(I/I_0) = -\int \mu(x, y) \, ds \]  (5.10)

where \( t \) is the distance of the line integral from the origin of the coordinate system and \( \theta \) is an angle between a normal line and \( x \)-axis. The meaning of noted parameters is depicted in Fig. 5.5. Line \( AB \) can be expressed as follows:

\[ x \cos \theta + y \sin \theta = t \]  (5.11)

Using the delta function the Eq. 5.10 and 5.11 can be rewritten [61] as:

\[ p(t, \theta) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} f(x, y) \delta(x \cos \theta + y \sin \theta - t) \, dx \, dy \]  (5.12)

where \( f(x, y) \) represents \( \mu(x, y) \). The function \( p(t, \theta) \) is known as the Radon transform of the function \( f(x, y) \). A projection is composed of a set of parallel line integrals with the constant \( \theta \). This is known as the parallel projection and is shown in Fig. 5.6-left. This type of projection is rather theoretical because common X-ray sources have a divergent X-ray beam. The projection which represents a X-ray point source with a divergent beam is called the fan beam projection (see scheme in Fig. 5.6-right) and its line integrals are measured along the fans. Collected Radon transformation data are known as a sinogram. In the sinogram space, the vertical axis represents the projection angle while the horizontal axis represents a detector channel (horizontal
5.4. TOMOGRAPHIC RECONSTRUCTION

Figure 5.5: Scheme of Radon transform: object $f(x, y)$ and its projection $p(t, \theta)$ for angle $\theta$

Figure 5.6: Scheme of a parallel projection (left) and a fan beam projection (right)

resolution of the detector). Therefore, one projection is represented in the sinogram as a line of the samples along the horizontal axis. Sinogram is called sinogram because the word “sinogram” originates from the fact that the projection of a single point (located outside the centre of projection) as the function of the angle is plotted by a sinusodial curve. The sinogram is a powerful indicator for the analyses of projection data and detection of its discrepancies.

The theory of the tomographic reconstruction is generally known as Fourier slice theorem (FST) also called the central slice theorem. This theorem relates with the fact that Fourier transform of a parallel projection of an object $f(x, y)$ taken at the angle $\theta$
equals a line in the two-dimensional Fourier transform of $f(x, y)$ obtained at the same angle. In other words, the Fourier transform of an object’s projection at any angle equals a line taken in the same orientation of the two-dimensional Fourier transform of the same object [62]. Implication of this theorem is obvious. From each projection the line in two-dimensional Fourier transforms of the object by performing of the Fourier transform on the projection can be obtained. Reconstruction of the object can be performed using a collection of the sufficient number (theoretically from the infinite number of angles) of projections over the range $0 \div \pi$ which fill the entire Fourier space. If the Fourier transform is obtained the object can be recovered itself by the inverse Fourier transform. Thus, the tomographic reconstruction process is composed of a series of one-dimensional Fourier transforms followed by the two-dimensional inverse Fourier transform. The application of the inverse Fourier formula also gives an explicit inversion formula for the Radon transform. According to inverse Radon transform it is possible to express $f(x, y)$ from Eq. 5.12.

5.4.2 Filtered backprojection

However the FST provides a pure analytical solution for tomographic reconstruction, a practical implementation requires a different approach. The main disadvantage of the FST is a fact that the sampling pattern produced by FST in the Fourier space is non-Cartesian. These samples have to be interpolated to a Cartesian coordinate but interpolation in a frequency domain is not straightforward as the interpolation in real space. An error produced on a single sample in Fourier space affects the entire image while an error in real space affects only the small region where the sample is located.

The most popular practical implementation or tomographic reconstruction based on FST is a filtered backprojection (FBP) algorithm. This algorithm is extremely accurate and amenable for fast implementation. In FBP algorithm the FST is a transform to a polar coordinate and the limits of the integration are rearranged. The algorithm can be used for various types of scanning geometries (parallel beam, fan beam etc.). There are two steps in the FBP algorithm: the filtering part and the backprojection part. The filtering part can be likened to simple weighting of each projection in the frequency domain. The filtering is used to eliminate an undesirable artifact, e.g. blurring (star-like artifacts) with are present in simple backprojection. The backprojection part is an equivalent to finding the elemental reconstructions of previously filtered data.
5.4.3 Iterative reconstruction

Previously described FBP reconstruction is an analytic approach in its basis. Each projection is weighted, filtered and backprojected, and with the last projection the reconstruction process is finished and reconstructed images are provided. Whereas images generated using FBP are final and are not improving, the basis of iteration approach is to generate initial estimation and this estimation is improved during the iteration process. The basic principle is simple and can be expressed for a two-dimensional object (due to simplicity but can easily be extended to the three-dimensional case) as follows. The two-dimensional vector $\mu$ represents an object and $p$ represents its measured projections. These two variables are linked by a system matrix $A$ and the error vector $e$:

$$p = A\mu + e$$  \hspace{1cm} (5.13)

The system matrix $A$ can be determined based on a system geometry, a focal spot shape and many other significant physical properties. Values of the error vector $e$ expresses measured noise. The reconstruction process is to estimate $\mu$ given the measuring vector $p$. The estimation process is controlled by requiring $\mu$ and $t$ to satisfy specified optimisation criterion. During the reconstruction procedure a set of estimation of $\mu^{(0)}, \mu^{(1)}, \cdots, \mu^{(n)}$ is generated iteratively to obtain the optimal estimation $\mu^{(s)}$ which is based on $p$. Thus for each iteration $j$ the quantity of $p^{(j)}$ is calculated:

$$p^{(j)} = A\mu^{(j)} + e$$  \hspace{1cm} (5.14)

Based on the comparison between the calculated projection $p^{(j)}$ (obtained by performing forward projection) and measured projection $p$ the estimation $\mu^{(j)}$ is modified for further iteration step to minimise the criterion. This step continues for all projections views. The iteration process is repeated until the convergence criterion is not reached. This type of the iterative algorithm is called the algebraic reconstruction technique (ART). The importance of iterative reconstruction technique is growing with increasing computed power because the iterative process is computationally expensive. The computational complexity can be reduced using several techniques such as ordered subsets expectation maximisation (OSEM) \[63\]. In OSEM, an algorithm divides the projection data into ordered subsets where each subset contains a set of regularly spaced projections. Thus the iteration process is performed only with a selected subset instead of all projections (the subset number is the speed-up factor).
5.5 Image filtering and segmentation

The goal of image filtering and segmentation is modification of the reconstructed image data to a suitable form for a FE model development. Filtering consists of a set of methods where each pixel is modified based on its scalar value or scalar values of the surrounding pixels (commonly used some weight function) and are useful for noise removing, blurring, finding edges, etc. Whereas the filtering process adjusts an image as whole, the image segmentation is the process of dividing an image into multiple parts. This is typically used to identify and select an objects of interest. Segmentation approaches can be generally divided to three categories [64]: thresholding, edge-based method, region-based method. In thresholding, pixels are allocated to categories according to the range of values in which a pixel lies. In edge-based method, pixels are classified depending on the previous identified edges, i.e., pixels which are not separated by an edge are assigned to the same category. The region-based approach iteratively grouping together pixels which are neighbours and have a similar scalar value. Some of the above mentioned approaches will be described in more detail below.

Filtering and image segmentation method must be used carefully because every change in the morphology of the object (growing thin walls, loss of connectivity etc.) can has a crucial effect on results in FE analyses. The application of inappropriate algorithms or algorithms with inappropriate properties can have undesirable influence on computed stress distribution.

5.5.1 Gaussian smoothing

The Gaussian smoothing is the most common type of a blurring filter which is used in digital imaging. The Gaussian smoothing operator is a convolution operator that is used for blurring images and removing detail and noise. Mathematically, applying the Gaussian smoothing to an image is equal to convolving the image with a kernel that represents shape of Gaussian function. For digital imaging the two-dimensional discrete convolution is used:

\[(f \ast h)(x, y) = \sum_{i=-k}^{k} \sum_{j=-k}^{k} f(x - i, y - j) \cdot h(i, j) \quad (5.15)\]

where \(s\) is the kernel and \(h\) is the original scalar value of the pixel. In other words, the scalar value of a pixel in the original image is affected by neighbouring pixels at a rate
that depends on the kernel size and pixel values distribution.

5.5.2 Image thresholding

Thresholding technique is the simplest and the most commonly used method of image segmentation which is based on a comparison of scalar value of a selected pixel with previously defined threshold value or range. Based on the result of the comparison the scalar value of the pixel is processed according to a selected criterion (no modification, change to the defined value etc.). In digital imaging, thresholding is usually used to create binary images where the values are limited to 0 and 1. The threshold range can be selected by a user or automatically (e.g. histogram-based).

5.5.3 Regional based method

Region growing algorithms have proven to be an effective approach for image segmentation. The algorithm starts from a seed region (one or more pixels) that is included in the object which will be segmented. The pixels neighbouring this region are evaluated to determine if they should also be considered a part of the object. Pixels are added to the region and algorithm iteratively adds new pixels satisfying the criterion which is used to decide whether a pixel should be included in the region or not. Several implementations of the region growing algorithm are available. These algorithms are different in the strategy of visiting neighbouring pixels and which type of connectivity is used to determine neighbours. For example, the connected threshold method is based on an interval of intensity values provided by the user. The algorithm selects those pixels whose intensities are inside the interval.

5.5.4 Region/volume of interest

The region of interest (ROI) or volume of interest (VOI) are general terms for selecting area (or volume) from whole image data for further processing. The part of the image data is selected usually using a tool with a polynomial shape (rectangular, circle, ellipse, box). It is a basic segmentation method for coarse reduction of working space of further filtering or segmentation algorithm and decreasing of computing complexity.
5.6 Type of microstructural models

From the viewpoint of element’s shape, two basic approaches of FE models are used: a geometry-based model and a voxel-based model. In the geometry-based approach the volume is discretised using tetrahedral elements which are capable to fill the whole volume without geometric simplification. Although the surface can not be refined to achieve full volume discretisation, it is usually smoothed before meshing due to elements number reduction. On the other hand, in a voxel-based approach the volume of the model is discretised using hexahedral elements and the real surface is only approximated by ‘cubes’. Influence of geometry approximation based on the type of elements on accuracy of FE analyses is discussed in many works [65]. In [66], the importance of a proper segmentation while minimal influence of an element type (tetrahedral vs. hexahedral) and resolution (above of limiting value) of based image data is demonstrated.

5.6.1 Tetrahedral models

Tetrahedral models are composed of tetrahedron elements (polyhedron composed of four triangular faces) and the whole volume of an object is theoretically discretised without surface simplification. Commonly, the first step of the tetrahedral model development is the surface reconstruction from spatial image data. For the surface reconstruction several approaches exist but historically the most known algorithm for extracting a polygonal mesh from three-dimensional scalar data is the Marching cube algorithm [57] published in the 1987 by William E. Lorensen and Harvey E. Cline. This algorithm divides the object using a three-dimensional grid and according to the grid virtual cubes are defined. Each vertex of the cube is evaluated in dependence on the scalar value of the pixel (outside or inside the object). The combination of evaluated vertex (2³ possible combination) determines how the cube will be divided by triangle(s). These generated triangles of all virtual cubes determine the surface (polygonal network) of the object. Generated polygonal mesh is usually optimised (e.g. Delaunay triangulation) and smoothed [67] to decrease a number of polygons. During the surface optimisation process it is necessary to control changes of the surface geometry because these modifications can have a significant influence on results in the
5.6. TYPE OF MICROSTRUCTURAL MODELS

FE analyses. Surface polygons are subsequently converted into tetrahedrons and the rest of the volume is also discretised by tetrahedrons. In this way the volumetric tetrahedral FE model is developed whose elements geometry strongly depend on the geometry of the modelled object. The quality of these elements (vertex angles) is crucial for convergence and accuracy of FE analysis and from this reason the meshing process must be carefully controlled.

5.6.2 Voxel models

Voxel models are composed of hexahedral elements. The main advantage of this type of the model is its relatively simple development. Hexahedral elements can be directly generated from source image data where each voxel (i.e. pixel which is extended by a third dimension corresponding to the distance between slices) is converted to one element. All elements are geometrically identical with a regular shape and can be directly used in any FE toolkit. Two types of hexahedral elements are commonly used for FE simulations: (i) 8-nodes with linear shape functions and (ii) 20-nodes with quadratic shape functions. Depalle [68] has proven that for FE analyses of mechanical behaviour of the trabecular bone the 8-node hexahedral elements should be used with care because the stress fields can be generally poorly described (e.g. the bending of trabeculae leads to displacement fields with large gradients). An obviously improper description depends on the number of elements characterising the cross-section of the investigated trabecula (∼ resolution of the model). On the other hand, mechanical properties of the whole sample can be successfully estimated using elements with linear shape functions as was proven in our studies [66, 69].

In comparison with tetrahedral models the surface of the voxel model is approximated with less accuracy which can has a significant influence on some type of analysis (computational fluid dynamics, contact analyses etc.). On the other hand for an inverse simulation of the compression test this influence is negligible. In the case of objects with a complex inner structure is often necessary to use voxel models due to computational complexity because the number of elements is significantly lower than in the case of tetrahedral ones. A comparison of tetrahedral and voxel model of the trabecular bone generated from the same image data is illustrated in Fig. 5.7.
5.7. MODEL DEVELOPMENT SOFTWARE TOOL

For purpose of this dissertation work, data segmentation and modelling software tool mainly for processing of image data obtained from tomography has been developed. The application was designed as open-source with an emphasis on simple extension using custom plugins. The core of the toolkit is created in Python programming language\(^1\) and Qt toolkit\(^2\) is used for the graphical user interface (GUI). The core performs data interchanging and compatibility between the plugins. Plugins are implemented as an independent tool for image processing and visualisation using the visualisation library VTK\(^3\). The application and all parts are open-source and cross-platform and are ported to Linux (tested), Windows (tested) and Mac (untested) platforms. The application includes plugins for import data, image filtering and segmentation, modelling, data visualisation and export data to format for its further processing in third-party applications. Currently implemented plugins are following:

- plugin for import data in HDF5 (Hierarchical Data Format\(^4\) ) from matlab and octave toolkit

  - plugins for visualisation

    - visualisation of 2D data (slices)

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1. [http://www.python.org](http://www.python.org)
2. [http://qt-project.org/](http://qt-project.org/)
4. [http://www.hdfgroup.org](http://www.hdfgroup.org)
– visualisation of 3D data (tetrahedral models, voxel models, volume rendering)

• plugins for image filtering
  – thresholding
  – ROI and VOI selecting
  – Gaussian smoothing
  – dilatation and eroding
  – regional-based segmentation
  – polygonal decimation and smoothing

• plugins for modelling
  – Marching cubes algorithm
  – Voxel modelling

• plugins for export data to HDF5, STL (stereolithography), APDL (ANSYS Parametric Design Language), VTK and VTI format, RAW format (ordered list of elements, nodes and its connectivity)

Demonstration of toolkit visualisation outputs (volume rendering, tetrahedral and voxel modelling) performed from input data obtained using the tomography scanning are shown in Fig. 5.8.

Figure 5.8: Demonstration of the developed toolkit for data segmentation and modelling: visualisation of rat vertebrae from segmented slice (left); tetrahedral and voxel model of porous aluminium metal foam (right)
Chapter 6

Compression test of the trabecular bone

6.1 Introduction

To verify the proposed material model for the trabecular bone which was identified using nano and micro-scale, a compression test was carried out. Identified constants were verified based on the comparison of 'real' deformation behaviour of the trabecular sample and deformation of the corresponding model calculated using the FE analysis. For this purpose a proper description of deformation of the sample during the compression test is essential. Traditional experimental techniques such as strain gauging [70, 71], shearography, speckle interferometry, thermal stress analysis and reflectometry are well understood and a reliable technique but they are limited to a few discrete points and restricted to the surface of the object. On the other hand, Digital Image Correlation (DIC) technique is a powerful tool to measure surface strain field and can be extended to three-dimensional space as Digital Volume Correlation (DVC). DVC performs image correlation of volumetric image datasets and it is promising approach to internal deformation mapping, particularly for larger displacements and strains frequently associated with failure processes. Practicability and accuracy of DVC are strongly dependent on quality of source image data and increased interest in this approach is linked to the improved availability of a high resolution CT. The possibility of computed tomography technique and principles lead to a volumetric image dataset and the FE model development have been introduced in previous chapter 5. In this work the high resolution micro-CT tomography was used to obtain volumetric image data of a trabecular sample
6.2 SAMPLE PREPARATION

in multiple deformation states during the compression test. Captured volumetric image data of the undeformed sample was processed to develop the FE model and data from all deformation states were used to track displacements of the structure and evaluate the inner deformation fields of the trabecular tissue. Evaluated deformation is further compared with calculated values assessed using the FE simulation of the compression test.

6.2 Sample preparation

A cylindrical sample of trabecular bone was drilled from the same human femoral head as in case of the nanoindentation test (see Fig. 6.1). Ends of the drilled sample were trimmed to achieve planparallel top and bottom surface. The cylindrical sample dimension after cutting was 5 mm in diameter and 17.2 mm in length. The sample was delipidated and rinsed with distilled water using ultrasonic bath and for speed up of the cleaning procedure airflow was used between ultrasonic cleaning cycles. Both ends of the cleaned sample were embedded into methylmetacrylat (VariKleer, Buehler GmbH, Germany) for elimination of the boundary artefacts caused by the sample preparation. Before the compression test the sample was stored in distilled water.

![Figure 6.1: Cylindrical sample prepared from the human femoral head (left); cleaned sample embedded into the methylmetacrylat and prepared for the compression test (right)](image.png)
6.3 Tomography of the compression test

To verify the proposed material model for the trabecular bone identified from the nanoindentation tests and the three-point bending, the compression experiment was carried out. Prepared cylindrical sample was placed into a special-purpose loading device. This loading device was specially designed for application in tomographic measurements and for this reason the bearing body (with constant 5 mm thickness) is made from a plexiglass with low X-ray attenuation. Load is controlled by a stepper motor (SX16, Microcon, Czech Republic) attached to a planetary gear unit and placed on a floating carrier. The load is applied to the sample through a translation stage (7T173-20, Standa Ltd., Lithuania) with 20 mm travel range attached with micrometric screw with 2 \( \mu \)m tracking accuracy. Reaction of the sample on an applied load is measured using an embedded load-cell (U9B, HBM GmbH, Germany) with 0 ÷ 500 N measuring range. Control of the loading device is performed using LinuxCNC tool on GNU/Linux real-time operating system. Main parts of the self-engineering loading device are depicted in Fig. 6.2.

![Loading device used to perform a compression test with description of the main parts](image)

Micro-CT measurement was performed using a unique radiographic setup situated at Laboratory of X-ray and neutron tomography of Centre of Excellence Telč (CET). The setup (see scheme in Fig. 6.3) includes fully motorized axes for variable distance
setting between X-ray source - sample - detector. This variability makes it possible to change the magnification of about 1.2 times to 100 times. Very stable high resolution is possible also with regard to the use of anti-vibration table, on which the whole assembly is placed.

![Scheme of the unique radiographic setup situated at Laboratory of X-ray and neutron tomography of CET.](image)

The loading device with a sample was placed on a precise rotational stage (APR150DR-135, Aerotech Inc., USA) to enable obtaining a suitable number of projections. The irradiation process of the sample was employed using high resolution tungsten X-ray tube (XWT 240 SE, X-ray WorX, Germany) with 4 µm focal spot size. Tomographic images were captured using flat panel X-ray image single substrate amorphous silicon active TFT/diode sensor (XRD1622, PerkinElmer Inc., USA) with the physical area (400 × 400 mm) corresponding to the sensitivity area 2048 × 2048 square pixels (200 µm pitch). Before the first load application, the tomography with 800 projections (corresponding to full rotation of the rotational stage) to achieve total 360° rotation of the unloaded sample was performed. Voltage and current on the tube during the tomography were set to 70 kV and 340 µA, respectively. Acquisition of one projection was set to 1 s. Setup geometry was arranged to obtain maximum possible magnification leading to source-sample distance 75 mm and source-detector distance 1335 mm. The resulting magnification was 17.8 × and one pixel of radiograph corresponded to 11.236 µm.

Projections of the unloaded sample (zero deformation state) were used to develop a
6.4 DATA RECONSTRUCTION

high resolution FE model. Consequent deformation steps were scanned with same parameters of voltage, current, number of projections and acquisition time as the zero deformation step. During the deformation steps the sample was incrementally loaded (loading rate 1 µm/s) with 1% overall strain increment up to total 6% deformation determined based on length of the sample. For relaxation of the sample structure before tomographic scanning a time delay (approximately 15 minutes) was inserted after the end of each loading phase. Tomographic setup for the compression test with loading device is depicted in Fig. 6.4.

Figure 6.4: Tomographic setup for the compression test

6.4 Data reconstruction

To improve quality of captured projections the set of the correction and calibration measurements with a geometry of setup same as in the case of the compression test were employed before a reconstruction process. Three type of measurement were performed: i) BH correction measurement; ii) dark current measurement; iii) flat field measurement. BH correction measurement was done with the set of aluminium calibrators with variable thickness (0.2, 0.4, 1, 2, 4, 10 mm) using the special designed revolver attached to a radiographic setup. Dark and flat field correction measurements were performed according to the theory described in section 5.3.

Improved projections were reconstructed to obtain spatial image data of each loading
6.5. DEFORMATION MAPPING USING DVC

state. Due to high porosity of the sample and small thickness of the trabeculae (approximately 200 µm), cone-beam reconstruction algorithm was used to eliminate the distortion of the reconstructed data caused by divergent nature of the X-ray beam. Reconstructed data were exported to HDF5 data format for further post-processing using DVC tool and the previously described model modeller (see section 5.7).

6.5 Deformation mapping using DVC

To verify the microstructural FE model and its ability to predict deformation behaviour it was necessary to measure the displacements in the loaded sample during the compression test. To establish the displacements (and strains) in the volume of the cylindrical sample, the tomographically captured load states were evaluated using DVC method. The DVC method employs tracking and image registration techniques known from 2D optical measurements (i.e. DIC, see section 4.3.2) to 3D to measure the change in the sequence of volumetric images. The DVC technique uses a maximisation approach to find the correlation coefficient for the best fit between subimages defined around control points established in the reference image. The correlation coefficient is determined by examining pixel intensity of the image subsets in the corresponding images and deformation mapping function is extracted. In this work, a nonlinear optimisation technique and an iterative approach to maximise the correlation coefficient was used. The cross-correlation coefficient \( r_{ij} \) is defined:

\[
    r_{ij}(u, v, \frac{\partial u}{\partial x}, \frac{\partial u}{\partial y}, \frac{\partial v}{\partial x}, \frac{\partial v}{\partial y}) = 1 - \frac{\sum_i \sum_j [I_0(x_i, y_j) - \bar{I}_0][I_1(x'_i, y'_j) - \bar{I}_1]}{\sqrt{\sum_i \sum_j [I_0(x_i, y_j) - \bar{I}_0]^2 \sum_i \sum_j [I_1(x'_i, y'_j) - \bar{I}_1]^2}}
\]

(6.1)

where \( I_0(x_i, y_j) \) is the pixel intensity in the reference (undeformed) image at a point \( (x_i, y_j) \) and \( I_1(x'_i, y'_j) \) is the pixel intensity at a point \( (x'_i, y'_j) \) in the consequent (deformed) image. \( \bar{I}_0 \) and \( \bar{I}_1 \) are the mean values of intensity in the images \( I_0 \) and \( I_1 \), respectively. It can be shown, that the coefficients of linear affine transformation between the undeformed and deformed state can be used to establish the components of displacement vector \( (u, v) \) and components of gradient tensor \( \frac{\partial u}{\partial x}, \frac{\partial u}{\partial y}, \frac{\partial v}{\partial x}, \frac{\partial v}{\partial y} \). Then, using the deformation gradient tensor \( \mathbf{F} \) it is easy to calculate the Green-Lagrangian strain tensor as:

\[
    \mathbf{E} = \mathbf{F}^T \mathbf{F} - \mathbf{I} = \frac{1}{2} \left[ (\nabla \mathbf{X} u)^T + \nabla \mathbf{X} u + (\nabla \mathbf{X} u)^T \cdot \nabla \mathbf{X} u \right]
\]

(6.2)
where $\nabla_XXu = \nabla_XX - I = F - I$ is the material displacement gradient tensor (partial differentiation of the displacement vector with respect to the material coordinates.

The calculation of an inner deformation of the sample through all loading states was performed using DVC algorithm implemented in Matlab. Due to computational complexity of DVC algorithm (to correlate the six deformation states for one point takes approximately 8.4 seconds using 4×Intel(R) Core(TM) i7-3820 3.60GHz CPUs) only nodes correspond to two mutually perpendicular planes ($I$ and $II$) passing through the centre of the sample were used to displacement evaluation. It was assumed that displacement of nodes in these planes was sufficiently informative about overall (and particularly local) deformation behaviour of the sample. Initial coordinates of nodes corresponding to these planes (15,550 and 16,032 nodes) were prepared from the FE voxel model of the intact state and their position were tracked in tomographic data (data were resampled by the same scale factor as in the case of the FE model, see section 7.2) through all deformation states.

### 6.6 Results and Discussions

Reaction force measured during the incremental loading is shown in Fig. 6.5. On
the left side of the image force-displacement dependency is shown while curve on the right side describes the force-time dependency. Due to relatively large relaxation of the sample a time pause (approximately 15 minutes) was inserted between the end of each loading phase and following tomography scanning. Tomographic scanning enabled to capture the inner deformation of the complex sample during compression test. Visualisation of deformation of the inner structure of the sample in the plane I and II is depicted in Fig. 6.6 and shows deformation through all six loading states with the 1% overall strain increment (calculated from the platen movement) and state at the end of the compression test where the trabeculae of the upper part of the sample were completely collapsed. These visualisations were useful for identification of a region of the interest for further DVC calculation.

Displacements of nodes in the plane I and II through all deformation states was tracked using DVC technique according to Eq. 6.1 and it is visualised in Fig. 6.7. Calculation of displacement of nodes corresponded to structure deformation depicted in visualisation in Fig. 6.6 proves the major deformation of the structure occurs in the upper part of the sample. Calculated values of displacement (850 ÷ 890 µm) in the upper part of the sample of the last loading step which should be corresponded to described platen movement (1032 µm) were significantly lower. This discrepancy was probably caused by improper boundary conditions on both ends of the sample. Methylmetacrylat caps were not completely parallel and lack of displacement could be caused by improper contact between the upper platen and the sample (this improper contact probably caused a slight bending of the sample). Determination of the correct movement of the structure bounded with the upper platen is crucial benefit of the DVC calculation because in the case of a displacement control of a FE simulation these 'real' values can be described to the FE model instead theoretical values derived from the actuator movement.

Green-Lagrangian (G-L) strain tensor of each hexahedral element was calculated from displacement of nodes according to Eq. 6.2. Field of G-L strain in plane I and II is shown in Fig. 6.8. Two regions of peak values were recognised in strain fields: i. a region I in upper part of the sample; ii. a region II in bottom correspond to the part of the sample embedded in methylmetacrylat. Values of strain in region II were affected by embedding compound and these values can not be correctly interpreted as strain values of pure trabecular bone structure. On the other hand the region I was not influenced by the sample preparation and strain values can be correctly interpreted. Plots confirmed a common assumption that the peak strain values are recognised in place of thin trabeculae and its connection to others. In the region I the
Figure 6.6: Visualisation of the deformation of the plane \( I \) (top) and \( II \) (bottom) obtained from reconstructed projections of states with overall strain increments: 0 \%, 1 \%, 2 \%, 3 \%, 4 \%, 5 \%, 6 \%. Last images show a total deformation of the sample at the end of the compression test.

strain values exceeded average strain value (theoretically 6\% overall strain) and this can be attributed to the fact, that major deformation of the sample occurs in the region \( I \). Based on this assumption and for precise strain description the DVC calculation in the full volume of the region \( I \) was calculated. DVC calculation of the displacement field (see Fig. 6.9) of the region \( I \) was very complex because movement of 185,156 nodes (115,182 elements) which approx. corresponded to 1/3 of the entire trabecular bone sample had to be computed.
6.6. RESULTS AND DISCUSSIONS

Figure 6.7: Vertical displacement of nodes (scale in µm) in plane I (top) and plane II (bottom) through all loading states calculated using DVC and mapped on the structure of the intact state

Resulting strain and displacement fields show the fact that sample was not subjected only to uniaxial compression - Fig. 6.9 illustrates bending of the sample. From cross-sectional views in Fig. 6.10 it is visible the rate of the bending in the inner structure. Non-homogenous displacement applied on the top of the sample was caused by non plan-parallelity of surfaces despite the fact that sample preparation was performed with emphasis on it. Although the further FE simulations should be designed as the perfect compression test, the DVC enables to perform the FE simulation more precisely, i.e, prescribes the full 3D displacement of the upper nodes of the FE model in displacement control simulation. Prescription of the real displacement to the FE model should alleviate inaccuracies in sample preparation process.

Average displacement value (in band corresponds to 1.7% of total height of the sample) of top nodes of the sample of both planes calculated using DVC was used to determine the overall strain. From geometrical dimensions and measured force the overall stress
was obtained and stress-strain diagram was derived. Resulting stress-strain diagram is shown in Fig. 6.11. The graph in Fig 6.11 plots comparison between strain evaluated using DVC technique and platen movement. As it was discussed above the strain values derived from platen movement were lower that values calculated using DVC. Each point of the curve corresponds to one tomographic loading state. However obtained curve is not perfectly smooth due to limited number of loading states but the trend of the curve is clearly visible.

From the slope of the stress-strain curve the overall elastic modulus $E = 0.140$ GPa was determined. This value is significantly lower than values known from literature. Zhou [72] measured elastic modulus of human trabecular bone sample (8.5 mm in diameter) for various anatomical sites: proximal tibia ($E = 0.756 \pm 0.402$ GPa, $BV/TV^1 = \ldots$

\footnote{Bone Volume over Total Volume}
6.6. RESULTS AND DISCUSSIONS

Figure 6.9: Calculated displacement (left) and G-L strain field (right) of the last loading state of 1/3 of volume of the sample using DVC algorithm.

Figure 6.10: Calculated G-L strain field of the last loading state in cross-section views: cross section cut by one plane (left); cut by two plane (right)

0.101 ± 0.0029), vertebral body (\(E = 0.329 \pm 0.091\) GPa, \(BV/TV = 0.091 \pm 0.016\)), femoral neck (\(E = 3.052 \pm 1.458\) GPa, \(BV/TV = 0.286 \pm 0.098\)) and Greater trochanter (\(E = 0.472 \pm 0.341\) GPa, \(BV/TV = 0.094 \pm 0.0032\)). Chevalier [73] also compressed human trabecular bone specimen (four specimens with 8.5 mm in diameter, 12 mm in length) of femural head (\(E = 0.632\) GPa, \(BV/TV = 0.161\); \(E = 1.168\) GPa, \(BV/TV = 0.228\); \(E = 0.820\) GPa, \(BV/TV = 0.144\); \(E = 2.483\) GPa, \(BV/TV = 0.296\)). These results show strong dependency of elastic modulus on porosity (or BV/TV ratio) of the sample, i.e., with increasing porosity the elastic modulus decreasing.

The sample tested in this study had porosity 0.749 (\(BV/TV = 0.251\)) and according to
the results published in [73] the elastic modulus should be in range 1.1 to 2.4 GPa. In comparison to the expected range the determined elastic modulus $E = 0.140$ GPa was very low. The low value of the elastic modulus was surprising and special attention was paid to uncover possible measurement error. Strain was assessed by two independent approaches with relatively similar results and it is assumed that strain was properly evaluated. To exclude possible errors of the loading device or the load cell (stress evaluation) the compression test (reference test in micro-CT) was repeated twice (control tests). Each control compression test was performed at different loading device and force sensor without micro-CT scanning. Strain of the samples was evaluated only in plane using a CCD camera and DIC technique. Results from control measurements (measured force and deformation of the sample) were similar to result of the reference compression test. Based on the control measurements the force curve of the reference test is correct and the measurement procedure was thus verified.

Another possible reason for low elastic modulus relates to a change of material properties due to tissue degradation in the period between harvesting and testing. To determine potential changes in material properties a part of the trabecular tissue extracted from the sample before the compression test and stored in the same conditions was nano-indented to obtain elastic modulus. Average value of the elastic modulus $15.07 \pm 4.54$ GPa assessed by control nanoindentation test was very similar to elas-
tic modulus from the first nanoindentation 15.39 ± 1.4 GPa and these results do not indicate major bone degradation.

After exclusion of measurement error and material degradation the author is not aware of any reason why the overall elastic modulus measured during the compression test was significantly lower than results published in literature. Although it is possible that the sample had an abnormal inner structure causing the lower stiffness this fact would be uncovered by the following inverse FE simulation.
Chapter 7

Numerical simulation of the compression test

7.1 Introduction

Nowadays, Finite Element (FE) method is one of the most commonly used numerical approach to analyse and predict the mechanical behaviour of bone structure. The accuracy of a FE model to predict mechanical behaviour depends on many parameters such as element’s size and type (number of integration points, order of interpolation function etc.), description of material behaviour and the boundary conditions [68]. Due to dependency on these parameters it is necessary to validate the FE model. The validation of the FE model is an important step how to ensure that simulations accurately reflect physical models and that the validated micro-CT FE models are suitable for reliable investigation of the pre and post yield behaviour of trabecular bone using FE analyses [74, 75]. Ideally FE models are validated by experimental data, i.e., numerical simulations of a performed experiment. The aim of this chapter is to simulate the previously performed compression test (see chapter 6) in order to verify a micro-CT FE model of trabecular bone.

7.2 FE model preparation

The reconstructed tomographic data of the intact state were used to develop the microstructural FE model of the trabecular sample for the simulation of the compression
test. Original reconstructed image data of $1024 \times 1024 \times 1496$ pixel resolution had to be resampled using discrete cosine transform method [76] to $205 \times 205 \times 299$ pixel resolution (0.2 scale factor in all directions) to lower the complexity of the obtained FE model for further computation. The model obtained from original data set would be composed from $50,736,191$ elements ($56,723,459$ nodes) and it would mean to solve approximately $150 \times 10^6$ unknowns. It would not be possible to solve such a complex model using our computation server ($8 \times$ Intel® Core™ i7-3820 CPU 3.60 GHz, 64 GB RAM) and for this reason the complexity of the model had to be reduced. This reduction was carefully controlled to avoid the change in porosity and stiffness of the FE model. In this study the BV/TV ratio of the FE model developed from original data and reduced data were 0.251 and 0.259, respectively. The reduced image data were used to develop microstructural voxel model using the in-house open source modelling tool which was described in more details in section 5.7.

The geometry of the voxel model was imported to general purpose FE code and each voxel was directly represented by 8-node hexahedral element. The volume of the model was discretized by using 431,626 linear elements including 633,162 nodes (see Fig. 7.1). Material model identified based on micro and nano-scale testing was used in following

![Figure 7.1: Voxel FE model of the trabecular bone prepared for FE analyse and detailed view on hexahedral elements](image)

FE simulation. As it was mentioned in section 3.4.2 the damage model depends on plastic strain induced in the element and DVC results show that significant strain values
were present only in the upper part of the sample. To decrease the computational cost of the FE simulation the damage model was applied only to 1/3 of the upper part of the FE model. Values of the material constants used for the FE model are summarised in Tab. 7.1 and correspond to average values of constants identified during the nanoindentation and the three-point bending test simulations.

<table>
<thead>
<tr>
<th>Material Constant</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastic modulus</td>
<td>11850 GPa</td>
</tr>
<tr>
<td>Poisson’s ratio</td>
<td>0.2</td>
</tr>
<tr>
<td>Yield stress</td>
<td>175 MPa</td>
</tr>
<tr>
<td>Tangent modulus</td>
<td>1850 MPa</td>
</tr>
<tr>
<td>Damage constant D1</td>
<td>0.6</td>
</tr>
<tr>
<td>Damage constant D2</td>
<td>28</td>
</tr>
<tr>
<td>Creep constant C1</td>
<td>5.85 x 10^{-18}</td>
</tr>
<tr>
<td>Creep constant C2</td>
<td>5.4</td>
</tr>
<tr>
<td>Creep constant C3</td>
<td>1.49</td>
</tr>
<tr>
<td>Creep constant C4</td>
<td>0</td>
</tr>
</tbody>
</table>

Loading of the model during the FE analyse was displacement driven and identical as in the compression experiment. The values of the movement of all 2,337 upper nodes of the FE model were derived from DVC results and displacement vector (values of movement between the tomographic loading state were linearly approximated) was applied to each node in every loading step. Nodes of the bottom surface of the FE model were fixed (rigid boundary conditions). The solution was divided to loadsteps and preconditioned conjugate gradient (PCG) solver was used.

### 7.3 Results a discussions

The upper nodes of the FE model were loaded by displacement field derived from the DVC calculation whereas nodes of the bottom surface were fixed. Overall stress was calculated based on the sum of the vertical reaction force of all fixed nodes in the bottom surface of the model and physical dimensions of cross-section. Strain was evaluated directly from the prescribed movement of the upper nodes. Resulting stress-strain diagram with the comparison to experimental data is plotted in Fig. 7.2. From resulting stress-strain diagram it is evident that stiffness and post-yield behaviour predicted by the FE simulation were quite different in comparison to experimental
values. Overall elastic modulus $E_{\text{FEM}} = 0.967$ GPa was assessed from the slope of the linear part of the curve. In contrast with the experimental value ($E_{\text{exp}} = 0.140$ GPa) the numerical elastic modulus was in expected range described in the literature. Yield stress $\sigma_{\text{yFEM}} = 11.34$ MPa and yield strain $\varepsilon_{\text{yFEM}} = 0.0137$ were evaluated using 0.2% offset method. Since, the measured elastic modulus was underestimated the comparison of predicted post-yield material constants with experimentally obtained values was irrelevant. Reasons why the experimental values could be significantly underestimated were discussed in section 6.6 but no measurement error or material degradation were detected.

Micro-CT scanning and DVC method enabled comparison of the deformation behaviour of the real compression test and its FE simulation at micro level. Nodes of the plane $I$ was localised in the FE model and their displacement and strain vector were compared with vectors evaluated using DVC method. Fig. 7.3 and 7.4 depict displacement and strain vector field in plane $I$ for third deformation step assessed using both method, i.e., FEM and DVC. These figures also show the residual displacement/strain field in vertical direction (z-axis) calculated as the difference between the DVC and the FEM field. From resulting residual displacement field it is evident that predicted displacement using the FE model is not uniform. In comparison to DVC displacement the FE displacement is larger in the upper section whereas in the bottom section it is lower. This phenomenon could be due to change in stiffness of trabeculae in middle and
7.3. RESULTS A DISCUSSIONS

Figure 7.3: Comparison of displacement field of the plane I for the third deformation step: DVC displacement field (left); FEM displacement field (middle); residual field of major Z displacement (right)

Figure 7.4: Comparison of strain field of the plane I for the third deformation step: DVC strain field (left); FEM strain field (middle); residual field (right)

bottom part of the sample. Compliance of the sample in these parts was significantly larger. This fact only confirms the conclusion derived from overall stress-strain curve and may indicate a material imperfection or induced damage in these parts of the sample. Strain fields in Fig. 7.4 correspond to resulting displacement fields, i.e., in the upper part of the sample the FE model predicted larger strain values (strain values
of the upper and the bottom ends of the sample are not relevant because they were affected by the embedding compound, see Fig. 6.6) while strain values in the middle and the bottom part were lower than in case of the real compression test.

Based on the results of the FE simulation it can be summarised that the FE model was unable to capture the performed compression test. However the predicted stiffness of the model was high (in comparison to experimental data) the value of overall elastic modulus was similar to value reported in literature. Since the elastic FE model behaviour did not correspond with measured values others parameters (yield stress, yield strain etc.) could not be reliably compared.

Despite the fact that proposed methodology was not reliably verified using described compression test this methodology was successfully applied in other cases. In paper [77] we published “preliminary study” of the work described in this study where the compression test was performed with similar human trabecular bone sample (physical dimension, harvesting location) but with older versions of the micro-CT setup and loading device. Compared to the material model used in this study only the elastic material model was considered in the FE analysis. Results from the comparison of the FE simulation and DVC showed that for small deformation the procedure (which was further elaborated in this study) the deformation behaviour of the trabecular bone was well simulated. We also published a study [78] focused on investigation of the mechanical properties of a rat vertebrae during compressive loading. Here, the methodology described in this doctoral thesis as combination of nanoindentation (material model development), compression test in the micro-CT device (data for the FE model development and DVC), DVC calculation of displacement field and FE simulation was successfully used for verification of calculated and measured overall stiffness of the rat vertebrae and displacement of its inner structure. The part of the methodology was also utilised for investigation of structurally similar materials. In other our study [79] in which the procedure of material model identification based on the inverse simulation of the nanoindentation test was employed to quantify size of the affected zone of micro-scale aluminium specimen whereas in [80] the inverse identification of the material model based on the three-point bending test was used for the determination of elastic and plastic constants of isolated cell walls of the metal foam. In spite of the fact that the compression test performed in chapter 6 was not reliable verified, application of the proposed methodology in several cases demonstrates the universality and ability of the procedure to predict deformation behaviour of the trabecular bone as well as the structurally similar samples.
Chapter 8

Summary

The thesis focused on the development of the procedure to obtain a microstructural model for porous structures for reliable description of their mechanical behaviour. Trabecular bone as a representative porous material was investigated in this study with the goal to develop a numerical model for the simulation of the deformation behaviour during loading.

Numerical material model was identified on two resolution levels: nano-scale and micro-scale. At nano-scale level the nanoindentation test and its FE simulation were carried out to identify the material constants of the elasto-visco-plastic material model with damage. Numerical model used in the fitting procedure was tested in a sensitivity study to obtain optimal combination of computational complexity and results accuracy. At micro-level the three-point bending test and its FE simulation were performed. The three-point bending test of a single trabecula was performed using unique modular loading setup and deflection was evaluated optically using DIC technique. Modified fitting algorithm from the simulation of the nanoindentation test was used to identify the material constants. Combination of these two testing methods the material model of the single trabecula was identified. Results and discussion from identification of the material model at the single trabecular level are summarised in sections 3.6 and 4.5.

In the second part of the study the material model identified at single trabecular level was applied on the FE model of the complex sample. This FE model was used to simulate of the compression test to verify its functionality to predict the deformation behaviour of trabecular bone. In chapter 6 the compression test in the unique micro-CT setup and loading device was described in detail. Combination of these two prototype devices enabled to capture the inner structure of the sample under loading. Acquired
data were processed using computational techniques and in-house built software described in chapter 5 to develop geometrical model of the complex structure of the sample. Reconstructed image data were also used for spatial displacement assessment using DVC technique.

Compression test was simulated using inverse FE simulation and results were compared with values calculated using DVC technique. Although comparison of FE model and DVC results of the compression test showed discrepancy between displacement and strain values obtained experimentally and from numerical simulation the possible causes of this discrepancy were uncovered and discussed in sections 7.3 and 6.6. Despite this fact the numerical model and the procedures proposed in this study which combine modern experimental and computational approaches lead to development of microstructural model for prediction of mechanical behaviour of trabecular bone and can be successfully used in structural FE analyses as it was discussed and proved at the end of the section 7.3.

Methods and results published in this doctoral thesis were part of the outputs (see the list of candidate’s publications relating to the doctoral thesis) of the project called “Morphometry and mechanical properties of trabecular bone assessed by methods of micromechanics and numerical modelling” (grant No. P105/10/2305) with a total budget 91,000 Euro and the project called “Determination of Structural and Mechanical Properties of Metal Foams Using Nanoindentation, Computer tomography and Microstructural FEM models” (grant No. P105/12/0824) with a total budget 321,000 Euro both provided by Czech Science Foundation. Project P105/10/2305 was finished at the end of 2013 and classified as excellent with results with international impact. Project P105/12/0824 will be finished at the end of 2014.
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