Volumetric spatial decomposition of porous microstructures

A framework for element based analysis of trabecular bone

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Martin Stauber
Summary

Bone mineral density measures are currently the standard for fracture risk assessment in osteoporotic patients. Nevertheless, since it was recognized that bone density leaves a rather large variation in bone strength unexplained, many attempts have been made to also include trabecular structure in the bone strength prediction. Consequently, many morphometric indices were devised to characterize trabecular bone architecture. Where most of these methods analyzed bone samples as a whole, in this thesis a new framework was devised and implemented to analyze bone samples on an elemental level. With this method, for the first time, trabecular bone structures could be spatially decomposed into their volumetric rod and plate elements. Based on this volumetric spatial decomposition two applications were presented. First, the extracted elements were analyzed using conventional morphometric measures, a method that was referred to as local morphometry. Second, the extracted elements were converted to idealized computational models for a fast and accurate prediction of bone mechanical properties.

Local morphometry revealed large differences in the rod/plate composition of the structures from different anatomical sites. It was suggested that the strength of dense plate-like structures was determined by a few “major elements” spanning through the whole structure, whereas the strength in loose rod-like structures was determined by the relative arrangement, quality and shape of a whole set of elements. These site differences were also reflected in different age-related remodelling mechanisms. Where in lumbar spine bone loss was mainly expressed as a loss of rods, in femoral head bone loss was expressed by perforation of large plates followed by a transformation of plates to rods and by loss of interconnecting stabilizing trabecular elements. It was also suggested that these interconnecting elements played a key role in fracture initiation in inhomogeneous bone samples. Furthermore, local morphometry was able to accurately predict bone mechanical properties. A multiple linear regression model combining mean trabecular spacing (Tb.Sp), mean slenderness of the rods (⟨Ro.Sl⟩), and the relative rod volume fraction (Ro.BV/BV) accounted for 90% of the variance in Young’s modulus as computed by conventional finite element
method. From these results it was concluded that local morphometry was a helpful tool to improve the understanding of bone quality and the relative importance of local structural changes with age and in the determination of bone strength.

Idealized computational models could be created directly from the volumetric spatially decomposed images by converting the trabecular elements to beams in a corresponding beam finite element model. Although these models were highly idealized, the apparent elastic properties of a set of human trabecular bone samples were equally well predicted by these models as compared to conventional voxel FE models ($R^2 = 0.97$). The big advantage of beam-FE models over conventional voxel based FE models is the tremendous reduction in number of elements which goes along with a tremendous reduction in computation time (up to a factor of $10^4$). The strong reduction in CPU time opens up ways for research that was not possible before, such as the routine assessment of mechanical properties of large bone specimens or even whole bones.

In conclusion, a new framework for element based analysis of trabecular bone structures was introduced. Local morphometry and idealized computational models may become important tools to explain age- and disease-related changes in bone quality and the competence of bone. With upcoming in vivo high-resolution imaging systems idealized computational model have the potential to become a standard in routine bone failure prediction.
Zusammenfassung


Chapter 1

Introduction

Bone

Bone is a specialized connective tissue that makes up, together with cartilage, the skeletal system. Bone serves three functions: (a) mechanical, support and site of muscle attachment for locomotion, (b) protective, for vital organs and bone marrow, and (c) metabolic, as a reservoir of ions, especially calcium and phosphate, for the maintenance of serum homeostasis, which is essential to life.

The macroscopic bone architecture is classically described using long bones as a model (Figure 1.1). A typical long bone shows two wider extremities called epiphyses, a more or less cylindrical shaft in the middle called midshaft or diaphysis, and a developmental zone between the two called metaphysis. In a growing long bone, the epiphysis and the metaphysis originate from two independent ossification centers. They are separated by a layer of cartilage, called the epiphyseal cartilage or growth plate. This layer of proliferative cells and expanding cartilage matrix is responsible for the longitudinal growth of bones. By the end of the growth period this layer is remodelled, becomes entirely calcified and is finally replaced by bone. The external part of the bones is formed by a thick and dense layer of calcified tissue, the cortex or compact bone, which in the diaphysis encloses the medullary cavity where the bone marrow is housed. Toward the metaphysis and the epiphysis, the cortex becomes progressively thinner, and the internal space is filled with a network of thin, calcified struts called trabeculae. These trabeculae are conventionally classified according to their shape as rods and plates. This porous bone type is the cancellous bone, also named spongy or trabecular bone (Figure 1.2). The spaces enclosed by these thin trabeculae also are filled with bone marrow and are in continuity with the medullary cavity of the diaphysis. The bone surfaces at the epiphyses that take part in the joint are covered with a layer of articular cartilage that does not calcify.
There are consequently two bone surfaces at which the bone is in contact with the soft tissues, an external surface, the *periosteal surface* and an internal surface, the *endosteal surface*. These surfaces are lined with osteogenic cells organized in layers, the *periosteum* and the *endosteum*. Cortical and trabecular bone are constituted of the same cells and the same matrix elements, but there are structural and functional differences. The primary structural difference is quantitative: 80% to 90% of the volume of compact bone is bone tissue (as opposed to marrow tissue), whereas only 15% to 25% of the trabecular bone is bone tissue (the remainder being occupied by bone marrow, blood vessels, and connective tissue). The result is that 70% to 85% of the interface with soft tissues is at the endosteal bone surface, which leads to the functional difference: the cortical bone fulfills mainly a mechanical and protective function, and the trabecular bone, a metabolic function. However, this classification is rather simplified and in certain bones such as the vertebral bodies, load is mainly carried by trabecular bone (adapted from [1]).
Osteoporosis

There are many metabolic bone diseases where one or both of the above mentioned functions are not accomplished properly anymore. One of those diseases is osteoporosis, which is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. Bone strength reflects the integration of two main features: bone volume fraction and bone quality referring to bone architecture, turnover, damage accumulation, and mineralization [2]. Before the age of 50, it affects only a few, whereas in old age, few are left without fractures due to age- or disease-related reduction of bone strength. It has been estimated that 4 in 10 white women age 50 years and older will experience a hip, spine, or wrist fracture sometime during the remainder of their lives (Figure 1.2) [3]. The risk for white men is lower due to their shorter life expectancy and lower fracture incidence rates. Due to the large size of the affected population and because of the devastating impact of osteoporotic fractures on morbidity, mortality, and on social costs, osteoporosis is recognized to be an important public health problem [4]. Death rates in patients with a hip fracture is 12% to 20% higher than in persons of similar age, race, and sex [5]. The total medical expenditures of osteoporotic hip-fractures, which is related with the highest medical costs, has been estimated for many countries [6-14]. On a global scale, it was estimated that the number of
Figure 1.3: Common fractures in osteoporosis.

Hip fractures occurring in the world each year will rise from 1.66 million in 1990 to 6.26 million by 2050 [15]. Thus, assuming a total annual cost of roughly US$ 30’000 per hip-fracture case [13], the global expenditures will rise from US$ 50 billion in 1990 to almost US$ 200 billion in 2050.

The increased use of densitometry throughout the last decades reflected a focus on bone density as the most important predictor of osteoporotic bone fractures. Significant correlations of apparent density and different mechanical properties of cancellous bone have been demonstrated for large populations using power law regressions [16-22]. Although many older persons may lose bone, as expressed by a decrease in bone density, not all develop fractures. The reason is that bone density is not the sole determinant of fracture risk. Neuromuscular function and environmental hazards, influencing the risk of fall, the force of impact as well as bone strength are equally important factors [23]. Bone mineral density, geometry of bone, microarchitecture of bone and quality of the bone material are all components that determine bone strength as defined by the bone’s ability to withstand loading. On average, seventy to eighty percent of the in vitro variability in bone strength is determined by its density [24]. On an individual basis, density alone accounts for 10% – 90% of the variation in the strength of trabecular bone [25]. This also means that 90% – 10% of the variation in strength cannot be explained by bone density. It has been shown that changes in trabecular morphology lead to a disproportionate decrease in bone strength [26]. For this reason, microstructural information must be included in the analysis to predict individual mechanical bone properties [27,28]. Preliminary

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<th>Type of fracture</th>
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<td>Hip (%)</td>
<td>17.5</td>
<td>6.0</td>
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<tr>
<td>Vertebra (%)</td>
<td>16.5</td>
<td>5.0</td>
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<tr>
<td>Wrist (%)</td>
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<td>Any of the three (%)</td>
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data have shown that predicting trabecular bone strength can be greatly improved by including architectural parameters in the analysis [29-33]. However, the relative importance of bone density, architecture and local tissue properties, in the etiology of bone fractures, an issue referred to as bone “quality”, is poorly understood.

**Quantification of bone microstructure**

To assess the contribution of microstructure to bone mechanical competence, conventionally relatively small bone samples have been extracted from the body, where it was proposed to either use cubic or cylindrical specimen geometry [34]. For the assessment of bone mechanical properties, different experimental testing methods have been proposed [35]. For trabecular bone, compression [20,22,32,34,36,37], and tensile testing [22,37] were used to assess apparent Young’s modulus and ultimate strength. Additional to these experimental methods, the stiffness and strength of trabecular bone samples was also computed by finite element model simulations [38-40], which showed to yield qualitatively similar results as experimental approaches.

The assessment of bone strength has traditionally been used to invent independent measures potentially predicting and explaining the variation in stiffness and strength, where the main focus was set on the characterization of bone microstructure. Conventionally, histomorphometry has been used to investigate bone structures. This method allows computing parameters such as bone volume density (BV/TV) and bone surface density (BS/TV). Model dependent algorithms were developed to calculate mean trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), number of trabecular structures (Tb.N), and other indices needed to better characterize bone architecture [41]. However, histomorphometry is a two-dimensional technique which does not allow to determine the real three-dimensional structure of trabecular bone. New imaging methods such as micro-computed tomography are attractive for such a characterization.

Micro-computed tomography (μCT) is a non-destructive imaging technique which allows to achieve images at a very high resolution (~10 μm). From two-dimensional projections a fully three-dimensional image can be reconstructed [42]. The advantage of three-dimensional images is that they enable assessment of truly three-dimensional parameters which may not be correctly computed from two-dimensional sections. Such indices can be used to estimate the influence of bone architecture on the mechanical competence of bone. Additionally, μCT is an ideal candidate to expand the classical mechanical protocols because it allows to monitor bone architecture and its behavior under loading in a time lapsed fashion due to its non-destructiveness. Müller et al [43] developed a new technique for nondestructive
three-dimensional assessment of local bone failure. They presented a compression device that fits in a commercial $\mu$CT scanner and allows to follow the deformation of a three-dimensional structure under compression. This method gives the possibility, for the first time, to acquire three-dimensional temporal images (4D-images) using animations.

The basic morphometric indices that can be derived from $\mu$CT images are the bone volume (BV), the bone surface (BS), and the total examined volume (TV). The bone surface can be obtained by calculating the surface of the triangulated structure [44,45]. Similarly the trabecular bone structure can be meshed volumetrically to determine its volume [46]. The basic measures of the relative bone volume density (BV/TV) and bone surface to bone volume ratio (BS/BV) can then easily be derived. Additional to these basic parameters the mean trabecular thickness (Tb.Th), the mean trabecular separation (Tb.Sp), and the trabecular number (Tb.N) are often determined parameters that can be computed directly from the three-dimensional image without an underlying model assumption [47]. To estimate the plate-rod characteristic of a trabecular bone structure a parameter called structure model index (SMI) was invented [48], which is 0 and 3 for an ideal plate structure and an ideal rod structure, respectively. It was shown that the SMI is closely related to mean curvature $\langle H \rangle$ of the bone surface [49]. Also related is the trabecular bone pattern factor (TBPf) [50]. It can be shown that this parameter equals $2 \langle H \rangle$ if extended to the third dimension. Another architectural index often used is the mean intercept length (MIL) characterizing the anisotropy of the structure [51]. Many researchers argued that trabecular bone is mainly anisotropic when comparing the anatomical loading axis to a perpendicular axis and approximately isotropic perpendicular to the anatomical axis, also referred to as transversely isotropic. To parameterize this behavior the tubularity (Tub), a measure for the bone’s directedness along the loading axis, and the transverse contiguity (Tcon), a measure for the distribution of the bone in transverse plane, were conceived. Both can be derived from autocorrelation functions in longitudinal and transverse directions, respectively [52]. Other measures of the extent of architectural anisotropy such as volume orientation, star volume distribution or star length distribution [53] were used to improve the prediction of multiaxial elastic properties of trabecular bone from bone volume density alone [54].

The methods mentioned so far all are an attempt to relate the properties of trabecular bone microstructure on a global basis to its mechanical competence. Only few attempts have been made to investigate truly local parameters of the trabecular network. Pothuaud et al [55] presented a method called line skeleton graph analy-
sis (LSGA) to compute topological parameters as well as the length and volume of single trabecular elements. They showed that LSGA can be applied \textit{in vivo} \cite{56} and has the potential to improve the prediction of mechanical properties when combined with bone volume fraction \cite{57}. Their method, however, was based on a line-skeleton where shape information was lost and an identification of plates and rods was not possible. An attempt to also assess shape information was done by Saha \textit{et al} \cite{58}, who first introduced a method for the digital topological characterization of the trabecular bone architecture. Their method is based on a thinning algorithm \cite{59} followed by a classification algorithm \cite{60} and allowed them to subdivide the trabecular structure into its rods and plates. This method was later used for orientation analyses of the trabecular bone network \cite{61} and it could be shown that the locally determined orientations better described anisotropy than MIL.

However, no investigations have been done that analyzed rod and plate elements in their full volumetric extent. From these analyses local tissue property variations could be captured on an elemental level and would allow investigating transformations from plate-like structures to rod-like structures based on true local information. Such examinations may improve our understanding of the relative importance of local structural changes in trabecular bone to its strength and may in future studies reveal differences in aging and disease related fractures as well as the effect of pharmacological intervention in the prevention of such fractures.

**Objectives of the thesis**

The aim of this thesis was to implement a framework for element based analysis of trabecular bone microstructures. This framework is founded on an algorithm to extract single rod and plate elements from a trabecular bone structure which may be subjected to further analysis or converted to idealized computational models. Specifically, the following questions were posed:

- Can trabecular bone microstructures be subdivided into their basic rod and plate elements for subjection to local morphometry?

- How do local morphometric indices vary within different anatomical sites, with age and what is their potential to explain bone strength?

- Can the rod and plate elements be used to implement an idealized computational model for fast and accurate prediction of trabecular bone competence?

To address these objectives, we proposed a new method for the volumetric spatial decomposition of trabecular bone structure into its basic rod and plate elements
(Chapter 2). Based on this new method we presented two applications; local morphometry (Chapter 3) and idealized computational models (Chapter 4). These three chapters which are followed by an overall conclusion give the main structure to this thesis. In more details, the following steps were carried out:

In **Chapter 2** a new method for the volumetric spatial decomposition of trabecular bone structures into its basic rod and plate elements was proposed. This method represents the foundation for the element based analysis of bone microarchitecture. The method was based on a skeletonization approach, where the skeleton of a three-dimensional bone structure was used to identify rod and plate elements that were subsequently expanded to their actual volume by applying an algorithm called multicolor dilation. The aim of the first study in this chapter was (1) to device and implement this new method, (2) to validate the method on computer generated models, and (3) to apply the method to human trabecular bone samples to investigate site differences in local morphometry, i.e. morphometry as applied to each individual element. To optimize the skeletons used in the volumetric spatial decomposition method, two input parameters are needed. Changes in these parameters influenced the final skeleton and in cascade the spatially decomposed structure and the derived morphometric indices. Thus, the aim of the second study in this chapter was (4) to assess the sensitivity of local morphometric indices on these two model parameters.

In **Chapter 3**, age related changes in local morphometric indices as well as the relation between these indices and mechanical competence of trabecular bone was investigated. For this, two studies on human trabecular bone samples were performed. The aim of the first study in this chapter was (1) to assess age-related changes of global and local morphometric indices, (2) age-related gender differences, and (3) age-related site differences in these indices. To analyze these relations a set of human trabecular bone autopsies were harvested from three different anatomical sites (femoral head, iliac crest, and second lumbar spine). With these samples, a co-variance analysis was performed to test for linearity with age as well as for age-related site and gender differences. The aim of the second study in this chapter was (4) to relate local morphometry to the mechanical properties of trabecular bone. The elasticity was assessed experimentally by uniaxial compression testing and computationally using the finite element method. In the third study of this chapter, an image guided method was used to qualitatively describe bone failure in uniaxial compression experiments. The aim of this study was (5) to show limitations of global morphometry in predicting bone mechanical properties and (6) to demonstrate the importance of having methods able to capture local structural variations.
In Chapter 4 a second application of the volumetric spatial decomposition method was presented; idealized computational models. The question was how accurate idealized specimen specific beam finite element models can predict bone elastic properties. The aim of the first study in this chapter was (1) to demonstrate the working principle of beam finite element models. Therefore, one aluminum alloy sample was converted to a beam finite element model, and the computationally derived stress-strain curve was compared to experimental results. As these results showed that the two stress-strain curves were in good agreement, a further study with human trabecular bone samples was conducted. The aims of the second study in this chapter were (2) to introduce a sophisticated method to convert trabecular bone samples into beam finite element models (3) to compare the results for speed and accuracy to conventional voxel finite element models.

References


Chapter 2

Volumetric spatial decomposition
2.1 Volumetric spatial decomposition of trabecular bone into rods and plates – A new method for local bone morphometry

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in press: Bone 2005

Abstract:
Bone microarchitecture is believed to play a key role in determining bone quality. We therefore present a new method for the volumetric spatial decomposition of trabecular bone samples into its basic elements (rods and plates). This new method is a framework for the element based description of bone microarchitecture. First, the newly developed algorithm was validated on computer-generated models. Then, it was applied to 328 human trabecular bone samples harvested from 70 donors at five different anatomical sites (calcaneus, femoral head, iliac crest, lumbar spine 2 and 4), which were previously scanned by micro-computed tomography. Standard three-dimensional morphometric algorithms were used to analyze the trabeculae on an individual basis with respect to their volume, surface and thickness. The results were statistically compared for the five sites. In this study it was possible for the first time to spatially decompose trabecular bone structures in its volumetric elements; rods and plates. The size of the largest element in the structures showed significant differences for the five compared sites. In samples from femoral head we found that basically one “major element” was spanning through the whole structure whereas in lumbar spine and calcaneus smaller elements dominate. From this we suggest that the strength of strong, dense plate-like structures is determined by the major elements whereas in looser rod-like structures the strength is given by the arrangement, quality and shape of a whole set of elements. Furthermore, we found that globally determined structural indices such as the mean curvature of the
bone surface $\langle H \rangle$) or related to this the structure model index (SMI) are almost exclusively explained by the arrangement of the plates. This also suggests that rods hold independent information characterizing trabecular bone quality, especially in the spine. These findings may improve the understanding of the site-specific role of bone microarchitecture in determining bone quality and in future studies the competence of bone.

**Keywords:**
Trabecular bone architecture, bone strength, bone quality, local morphometry, volumetric spatial decomposition

**Introduction**

Osteoporosis, is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. Bone strength reflects the integration of two main features: bone mineral density expressed as grams of mineral per area or volume and bone quality referring to bone architecture, turnover, damage accumulation, and mineralization [1]. Thus it is not unexpected that older persons may lose bone, as expressed by a decrease in bone density, but do not develop fractures. Bone mineral density, geometry of bone, micro-architecture of bone and quality of the bone material are all components that determine bone strength as defined by the bone’s ability to withstand loading [2,3]. For this reason, micro-structural information must be included in the analysis to predict individual mechanical bone properties [4,5].

Bone micro-architecture can easily be assessed *in vitro* by means of micro-computed tomography ($\mu$CT) [6] and in peripheral regions *in vivo* by quantitative-computed tomography (QCT) [7,8] and magnetic resonance imaging (MRI) [9-11]. In this paper we will concentrate on images acquired with $\mu$CT, a non-destructive technique with high spatial resolution. The basic morphometric measures are the relative bone volume density (BV/TV) and bone surface to bone volume ratio (BS/BV). Additional to these basic parameters the mean trabecular thickness (Tb.Th), the mean trabecular separation (Tb.Sp), and the trabecular number (Tb.N) are often determined parameters that can be computed directly from the three-dimensional image without an underlying model assumption [12]. To estimate the plate-rod characteristic of a trabecular bone structure a parameter called structure model index (SMI) was invented [13], which is 0 and 3 for an ideal plate
structure and an ideal rod structure, respectively. It was shown that the SMI is closely related to mean curvature \( \langle H \rangle \) of the bone surface [14]. Also related is the trabecular bone pattern factor (TBPf) [15]. It can be shown that this parameter equals \( 2 \langle H \rangle \) if extended to the third dimension. Mean intercept length (MIL) and other measures of the extent of architectural anisotropy such as volume orientation, star volume distribution or star length distribution [16] were used to improve the prediction of multiaxial elastic properties of trabecular bone from bone volume density alone [17]. Using finite element models the apparent stiffness, which is an important inherent attribute of trabecular bone samples can be computed directly and very accurately [18,19].

The methods mentioned so far all are an attempt to relate the properties of trabecular bone microstructure on a global basis to bone quality. Only few attempts have been made to investigate truly local parameters of the trabecular network. Pothuaud et al [20] presented a method called line skeleton graph analysis (LSGA) to compute topological parameters as well as the length and volume of single trabecular elements. They showed that LSGA can be applied \textit{in vivo} [21] and has the potential to improve the prediction of mechanical properties when combined with bone volume fraction [22]. Their method however, was based on a line-skeleton where shape information was lost and a identification of plates and rods was not possible. An attempt to also assess shape information was done by Saha et al [23], who first introduced a method for the digital topological characterization of the trabecular bone architecture. Their method is based on an thinning algorithm [24] followed by a classification algorithm [25] and allowed them to subdivide the trabecular structure into its rods and plates. This method was later used for orientation analyses of the trabecular bone networks [26] and it could be shown that the locally determined orientations better described anisotropy than MIL.

In this study we present a new approach, conceptually combining the three-dimensional identification of trabecular elements [20] with the classification of shape preserved skeletons [25]. In contrary to earlier studies we do not analyze the skeleton but present a method to decompose the trabecular structure into its basic elements (i.e. rods and plates) in its full three-dimensional and volumetric extent. This enables to compute morphological parameters for each element within the trabecular bone structure allowing for the first time to investigate truly local morphometric parameters of the trabecular bone network for rods and plates separately. The specific goals of this study were to (1) present the new volumetric spatial decomposition algorithm, (2) to validate this algorithm, and (3) to use the method for the morphometric description of site specific differences.
Methods

This section is structured in three parts. In the first part we describe the newly introduced algorithm to spatially decompose porous structures such as trabecular bone into its underlying elements. The second part shows three mathematical models that were used for the verification of the algorithm. Finally, in the third part, we applied the new algorithm to a set of 328 human trabecular bone structures from five different anatomical sites, which are calcaneus, femoral head, iliac crest, second lumbar spine, and fourth lumbar spine. These samples have previously been described as part of the European Union BIOMED I Concerted Action “Assessment of Bone Quality in Osteoporosis” [27,28].

The spatial decomposition of trabecular bone

In this section we present a new method for the spatial decomposition of trabecular bone into plate and rod elements. For this we need a binary three-dimensional image, where bone is separated from other tissues and background, as a starting point. A detailed description on digital topology in three dimensions can be found elsewhere\(^1\) [29-32].

In this paper we present a skeletonization approach, where we use the term skeletonization for an algorithm that transforms a 3D binary image into a new 3D binary image, denoted as skeleton, that has the same topology and shape information but which is only one voxel thick. Although this definition seems to determine the skeleton of an object pretty well, there are infinite ways one can think of on how to compute such a skeleton and many different approaches have already been proposed [24,33-43]. In this paper, we used a series of algorithms to compute the final skeleton. In a first step, we computed a very rough two voxel thick skeleton using the MB-3D algorithm proposed by Manzanera \textit{et al} in 2D [36], 3D [33,37] and nD [39]. This algorithm is computationally fast, topology- (homotopic) and shape-preserving.

In order to reduce the MB-3D skeleton\(^2\) to a one voxel thick skeleton we introduce a new algorithm, subsequently called \textit{conditional erosion (CE-3D)}. It is a fully parallel algorithm that can be applied to any two-voxel-thick skeleton. To remove all dispensable points, the algorithm runs in 6 subsequent scans whereby the structuring element \(\gamma_i\) is rotated in all 6 possible directions. In a first step, all points that

\(^1\)A few mathematical definitions, which are not part of the original publication, are provided in Appendix A.

\(^2\)A more detailed description of the MB-3D and the CE-3D algorithms, which is not part of the original publication, is provided in Appendix B.
2.1 Volumetric spatial decomposition of trabecular bone

Figure 2.1: Structuring elements used by the CE-3D skeletonization algorithm. The transparent cubes are set to background and the light grey cubes are set to the image. The dark grey cube in the structuring element $\gamma_1$ marks the center of the structuring element.

contain the structuring element $\gamma_i$ are marked and stored in a separate image $C$. In a second step all points that are needed to maintain topology are stored in image $D$. Those points are detected by scanning the 3x3x3 neighborhood of each point for the structuring elements $\delta_i$ ($i = 1, 2$), where a point is marked, if $\delta_i$ is contained in its 3x3x3 neighborhood. The third step removes then all points that belong to $C$ but not to $D$. This algorithm is performed only once. The three structuring elements needed for this algorithm are shown in Figure 2.1.

Analyzing these two first procedures results in a one-voxel-thick skeleton of the original image. However, this skeleton is very rough and needs optimization. In a first step we used a point-classification algorithm that is able to compute for each voxel whether it is a surface point, a surface end point, an arc point, an arc end point, an arc-arc intersection point, an arc-surface intersection point, a surface-surface intersection point or an isolated point. The basic principle of this algorithm was introduced by Saha and Chaudhuri and is described in details elsewhere [25]. However, we had to modify this algorithm slightly since our skeletons were not ideal. Especially intersection points could not be detected using the original algorithm.

To the classified skeletons we then applied two optimization procedures. The first was designed to reduce slender planes, which may appear from rod-like elements with elliptical cross-section, to arcs. To achieve this goal we removed iteratively all surface-end points, which means that a one-voxel thick boundary was removed from all surfaces. In each iteration the skeleton was newly classified to retrieve a properly classified skeleton. For the optimization we used a parameter which we would like to call the slenderness parameter $s$. This parameter is simply the number of iterations applied to reduce the surfaces. The second algorithm was invented to remove end-arcs (an end-arc is an arc where one node is an arc end point) arising from

\footnote{A more detailed description of the point-classification algorithm, which is not part of the original publication, is provided in Appendix C.}
surface noise. For this purpose each rod which was shorter than a critical length was removed from the skeleton. The critical length defines a new parameter which we would like to call noise parameter n. A sensitivity study of these two model parameters showed that the noise parameter n had only a minor effect on the skeleton when set to twice the value of the slenderness parameter s, which leaves the model with one single optimization parameter s. In this study, the model parameter s was set to ten, which proved to yield in visually reasonable results for all computer generated models (Figure 2.3) as well as for all human trabecular bone samples at all sites (Figure 2.4). The elements in the computer models were perfectly identified independent of their relative size and thickness. Also the inspection of the decomposed human bone structures showed good results in all sites using this parameter for optimization. Additionally, this value was supported by the sensitivity analysis, where we found that, although decomposition was sensitive to the parameter setting, the derived morphometric indices were well behaved where an increase of s by one caused an average change in local morphometric indices of about 1.8%. However, this parameter may have to be adapted when changing to a different resolution or a different species, similar to the settings for the Gaussian filtration and the global threshold used to binarize the images.

The image processing steps from the original image up to the optimized skeleton are visualized in Figure 2.2, where step A shows the original specimen and B shows the corresponding skeletonized and optimized point classified step.

This optimized skeleton served as the basis for spatial decomposition. The principal idea was to decompose the skeleton into its arcs and plates by identifying the elements in-between the intersection points. Arcs were defined as all arc-type voxels bound by two voxels being either an arc-arc intersection point, an arc-end point, or an arc-surface intersection point. Plates were defined as the points bound by surface-end points, surface-surface intersection points, and arc-surface intersection points. These arcs and surfaces were later used for the discrimination of rods and plates. After the thinning and optimization procedures, configurations could appear where two nodes were very close to each other. The interconnecting arc was then only a few voxels in length and it was therefore reasonable to remove this artificial arc and to identify these two nodes as one single node. To achieve this we deleted all voxels around the intersection points within a sphere of radius two. After this operation single elements in the skeleton could be identified and were written to a new image, where each such element was assigned a number. We used even numbers for the plate-like elements and odd numbers for the rod-like elements. This procedure is visualized in Figure 2.2C, where each element has a different color.
In a last step we had to identify the volumetric extend of the elements in the spatially decomposed image. For this purpose we used a dilation-related algorithm. The image was scanned alternating in forward and backward direction in order not to favor one direction only. In each scan a morphological dilation with a 26-neighbor structuring-element was applied and the new voxels were set to the color (or number) of the current voxel, where the new voxel could only be set if it was not already

Figure 2.2: Spatial decomposition of trabecular bone. The initial binary image that served as input for our algorithm is shown in (A). A skeletonization and optimization algorithm is applied to get a homotopic shape preserving skeleton as shown in (B). This skeleton is then point-classified, thus arc-, surface-, border-, and intersection-points are shown in different colors. (C) This point-classified skeleton is then spatially decomposed by removing the intersection points. (D) A two-way multicolor dilation algorithm was applied to find the volumetric extend of each element, yielding in the final spatially decomposed structure.
occupied by a neighboring element. The alternating forward-backward scanning also prevented that one element could grow on expense of a neighboring element and resulted in logical element-interfaces. We would like to call this procedure multicolor dilation. Additionally, to maintain the overall shape and volume of the structure, voxels were only set if they were also present in the original binary image. This operation yielded in a spatially decomposed image of the original structure where each element was assigned a number. Elements arising from arcs were identified to be rods and elements arising from surfaces were identified to be plates. The visualization of this last step is done in Figure 2.2D. The different numbers of the elements are visualized by different colors in the image.

Table 2.1: Dimension of the elements composing the three computer generated models shown in Figure 2.3.

<table>
<thead>
<tr>
<th>Model-Element</th>
<th>$R_A$</th>
<th>$R_B$</th>
<th>$R_C$</th>
<th>$R_D$</th>
<th>$M_A$</th>
<th>$M_B$</th>
<th>$M_C$</th>
<th>$P_A$</th>
<th>$P_B$</th>
<th>$P_C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>diameter (voxel)</td>
<td>11</td>
<td>21</td>
<td>21</td>
<td>11</td>
<td>–</td>
<td>–</td>
<td>9</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>length (voxel)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>181</td>
<td>181</td>
<td>71</td>
<td>181</td>
<td>181</td>
<td>121</td>
</tr>
<tr>
<td>width (voxel)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>181</td>
<td>181</td>
<td>–</td>
<td>181</td>
<td>181</td>
<td>71</td>
</tr>
<tr>
<td>thickness (voxel)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>9</td>
<td>9</td>
<td>–</td>
<td>9</td>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>

In all models the voxels were chosen to be 10x10x10 µm³.
Figure 2.4: The spatially decomposed trabecular bone structures of a 37-year-old man from (A) FH – femoral head, (B) IC – iliac crest, (C) CA – calcaneus, (D) L2 – second lumbar spine, and (E) L4 – fourth lumbar spine.
Local morphometry

Such spatially decomposed structures may serve as a basis for many new investigations and subsequent algorithms. In this study we used them to compute local morphometric indices on a trabecular level, i.e., morphometry as applied to individual rods and plates as identified directly from the three-dimensional images. In order to follow the naming conventions proposed by Parfitt et al [44] we suggest to use the prefix Pl for plates, Ro for rods, and El for elements that could be either plate or rod. The volume, surface, and thickness of one single element is denoted with V, S, and Th, and the sum of the volume and surface over all elements with BV, and BS, respectively. Furthermore, we use brackets (⟨⟩) to denote mean values that are averaged over all elements of the same type.

Basically all standard three-dimensional morphometric algorithms may be applied to the elements. In this paper we concentrated on only a few parameters which were the mean volume (⟨Ro.V⟩, ⟨Pl.V⟩), the mean surface (⟨Ro.S⟩, ⟨Pl.S⟩), as well as the mean thickness (⟨Ro.Th⟩, ⟨Pl.Th⟩) averaged for each structure over all rods and plates separately. Furthermore, the plate volume density (Pl.BV/TV), which is defined as total plate volume divided by total volume of interest in percent and the rod volume density (Ro.BV/TV), which is defined as the total rod volume divided by total volume of interest in percent, as well as the relative bone volume fraction of plates (Pl.BV/BV) and rods (Ro.BV/BV = 100% − Pl.BV/BV) in percentage, were determined. Another parameter investigated was the relative size of “major elements” (El.V_{max}/BV), which is defined as the volume of the largest element divided by total bone volume. All parameters were computed using standard morphometric algorithms as implemented in the image processing language IPL (Version 4.28d; Scanco Medical AG, Switzerland).

Validation of the algorithm on computer generated images

For the validation of the algorithm three computer generated models representing a rod-like (R), a mixed (M), and a plate-like (P) structure were produced. The three models are shown in Figure 2.3 and the dimensions of their elements are given in Table 2.1. The edge-length of a voxel was chosen to be 10 μm in each direction in order to present typical dimensions as found in trabecular architectures.

Each model was spatially decomposed with the newly developed algorithm, and the mean volume (⟨El.V⟩), surface (⟨El.S⟩), and thickness (⟨El.Th⟩) was computed for each element using standard 3D morphometry. For each element type, the mean value and standard deviation of those indices was then computed. The same indices
Table 2.2: Morphometric and analytic results of three computer generated models used for the validation of the algorithm. The table shows the mean values and standard deviations of the locally determined morphometric results as well as the analytic results determined for the three computer generated models shown in Figure 2.3. The labeling of the elements correspond to the labeling of the elements in Figure 2.3.

<table>
<thead>
<tr>
<th>Model-Element</th>
<th>( \langle El.V \rangle )</th>
<th>( R_A )</th>
<th>( R_B )</th>
<th>( R_C )</th>
<th>( R_D )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \langle El.V \rangle ) meas.</td>
<td>10^6 ( \mu m^3 )</td>
<td>7.56 ± 0.09</td>
<td>29.4 ± 0.0</td>
<td>30.7 ± 0.02</td>
<td>3.82 ± 0.05</td>
</tr>
<tr>
<td>( \langle El.V \rangle ) anal.</td>
<td>5.57 – 8.60</td>
<td>26.2 – 33.1</td>
<td>26.2 – 33.1</td>
<td>2.39 – 3.85</td>
<td></td>
</tr>
<tr>
<td>( \langle El.S \rangle ) meas.</td>
<td>10^3 ( \mu m^2 )</td>
<td>2.84 ± 0.02</td>
<td>5.84 ± 0.00</td>
<td>6.69 ± 0.02</td>
<td>1.51 ± 0.01</td>
</tr>
<tr>
<td>( \langle El.S \rangle ) anal.</td>
<td>2.47 – 3.13</td>
<td>5.52 – 6.30</td>
<td>6.13 – 6.94</td>
<td>1.42 – 1.82</td>
<td></td>
</tr>
<tr>
<td>( \langle El.Th \rangle ) meas.</td>
<td>( \mu m )</td>
<td>87.7 ± 0.0</td>
<td>189.6 ± 0.0</td>
<td>189.4 ± 0.0</td>
<td>87.6 ± 0.3</td>
</tr>
<tr>
<td>( \langle El.Th \rangle ) anal.</td>
<td>90 – 110</td>
<td>190 – 210</td>
<td>190 – 210</td>
<td>90 – 110</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model-Element</th>
<th>( \langle El.V \rangle )</th>
<th>( M_A )</th>
<th>( M_B )</th>
<th>( M_C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \langle El.V \rangle ) meas.</td>
<td>10^6 ( \mu m^3 )</td>
<td>294 ± 0</td>
<td>294 ± 0</td>
<td>3.42 ± 0.00</td>
</tr>
<tr>
<td>( \langle El.V \rangle ) anal.</td>
<td>224 – 295</td>
<td>224 – 295</td>
<td>2.73 – 4.52</td>
<td></td>
</tr>
<tr>
<td>( \langle El.S \rangle ) meas.</td>
<td>10^3 ( \mu m^2 )</td>
<td>70.9 ± 0.01</td>
<td>69.8 ± 0.00</td>
<td>1.74 ± 0.00</td>
</tr>
<tr>
<td>( \langle El.S \rangle ) anal.</td>
<td>68.5 – 71.7</td>
<td>67.9 – 71.3</td>
<td>1.52 – 2.01</td>
<td></td>
</tr>
<tr>
<td>( \langle El.Th \rangle ) meas.</td>
<td>( \mu m )</td>
<td>90.0 ± 0.0</td>
<td>90.0 ± 0.0</td>
<td>69.5 ± 0.0</td>
</tr>
<tr>
<td>( \langle El.Th \rangle ) anal.</td>
<td>70 – 90</td>
<td>70 – 90</td>
<td>70 – 90</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model-Element</th>
<th>( \langle El.V \rangle )</th>
<th>( P_A )</th>
<th>( P_B )</th>
<th>( P_C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \langle El.V \rangle ) meas.</td>
<td>10^6 ( \mu m^3 )</td>
<td>305 ± 0</td>
<td>294 ± 0</td>
<td>90.8 ± 0.1</td>
</tr>
<tr>
<td>( \langle El.V \rangle ) anal.</td>
<td>259 – 331</td>
<td>259 – 33</td>
<td>84.0 – 105</td>
<td></td>
</tr>
<tr>
<td>( \langle El.S \rangle ) meas.</td>
<td>10^3 ( \mu m^2 )</td>
<td>69.5 ± 0.00</td>
<td>64.4 ± 0.0</td>
<td>14.7 ± 0.0</td>
</tr>
<tr>
<td>( \langle El.S \rangle ) anal.</td>
<td>65.1 – 68.8</td>
<td>61.1 – 65.6</td>
<td>17.7 ± 18.7</td>
<td></td>
</tr>
<tr>
<td>( \langle El.Th \rangle ) meas.</td>
<td>( \mu m )</td>
<td>90.3 ± 0.1</td>
<td>90.4 ± 0.0</td>
<td>109.5 ± 0.0</td>
</tr>
<tr>
<td>( \langle El.Th \rangle ) anal.</td>
<td>70 – 90</td>
<td>70 – 90</td>
<td>90 – 110</td>
<td></td>
</tr>
</tbody>
</table>

were also computed analytically and the results were compared to the morphometric data (Table 2.2). For the analytic solution, dimensions of the elements ranged from a maximal to a minimal size. This was due to fact that different mathematically defined objects may represent the same digital object. A digital cylinder of 11 voxels (110 \( \mu m \)) in diameter, for instance, may be represented by all mathematically defined cylinders with diameters in the range of 9 to 11 voxels (90 to 110 \( \mu m \)).
Anatomical data of human trabecular bone

In order to test the newly developed algorithm on real structures, we applied our method to the large dataset of human trabecular bone samples from the European Union BIOMED I Concerted Action “Assessment of Bone Quality in Osteoporosis” [27,28]. The analyzed dataset encompasses 328 samples harvested from 70 donors (32 females, 38 males with ages ranging from 23 to 92 years (mean 69.4, SD 15.4 years). The samples were harvested from five different anatomical sites; femoral head (FH, n=64), iliac crest (IC, n=59), calcaneus (CA, n=69), second lumbar spine (L2, n=67), and fourth lumbar spine (L4, n=69). All samples were scanned using a micro-computed tomography (μCT) system (μCT 20, Scanco Medical AG, Switzerland) providing a spatial resolution of 28 μm. This system and scanning procedure is described elsewhere in detail [6]. The dataset has previously been used for several investigations [27,45]. A 4 mm cubic region of interest (TV) was selected from all samples. Using Gaussian filtration and global thresholding [27], binary images were created presenting either bone or background. A component labeling algorithm was applied to the binary images to remove all parts not 6-connected to the main structure. These images served then as the basis for the proposed spatial decomposition algorithm, which was applied using an optimization parameter of 10. For visual inspection of the outcome of the proposed algorithm, spatially decomposed structures of the five anatomical sites of an arbitrary donor (37-years-old man) were visualized (Figure 2.4).

The spatially decomposed structures have been analyzed using the GNU statistical computation and graphics package R (Version 1.7.1; http://www.r-project.org). For each structure we averaged the morphometric indices; for all rods, and for all plates (Table 2.3, Figures 2.5). For each morphometric index the mean values of all structures were compiled in a box-plot where the five site-groups were compared. Box-plots show the median value enclosed by a box holding 50% of the data spanned by the first and the third quartile. The whiskers show the range of the data, except some outliers that are shown separately by a small circle. The five groups were compared for significant differences \((p < 0.05)\) using a pairwise t-test with Bonferroni correction for multiple testing. If two groups did not differ significantly from each other they were assigned the same letter (e.g. A, B, . . .), which was also plotted in the graph. Thus, groups with the same letter did not differ significantly where groups not having the same letter did.
Table 2.3: Local morphometric indices of 328 human trabecular bone structures.

<table>
<thead>
<tr>
<th>Index</th>
<th>Site</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>⟨Ro.V⟩</td>
<td>FH</td>
<td>6.63</td>
<td>1.59</td>
<td>24.0%</td>
<td>6.45</td>
<td>3.16</td>
<td>10.34</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>6.14</td>
<td>2.05</td>
<td>33.3%</td>
<td>5.93</td>
<td>2.04</td>
<td>12.85</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>8.15</td>
<td>1.82</td>
<td>22.3%</td>
<td>7.71</td>
<td>5.15</td>
<td>14.30</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>5.69</td>
<td>1.60</td>
<td>28.1%</td>
<td>5.59</td>
<td>2.44</td>
<td>9.90</td>
</tr>
<tr>
<td></td>
<td>L4</td>
<td>7.54</td>
<td>1.94</td>
<td>25.7%</td>
<td>7.08</td>
<td>3.33</td>
<td>11.82</td>
</tr>
<tr>
<td>⟨Ro.S⟩</td>
<td>FH</td>
<td>256</td>
<td>41</td>
<td>16.2%</td>
<td>253</td>
<td>159</td>
<td>347</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>243</td>
<td>57</td>
<td>23.6%</td>
<td>240</td>
<td>113</td>
<td>414</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>301</td>
<td>42</td>
<td>14.1%</td>
<td>299</td>
<td>231</td>
<td>444</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>234</td>
<td>46</td>
<td>19.6%</td>
<td>235</td>
<td>129</td>
<td>349</td>
</tr>
<tr>
<td></td>
<td>L4</td>
<td>283</td>
<td>48</td>
<td>17.2%</td>
<td>276</td>
<td>164</td>
<td>392</td>
</tr>
<tr>
<td>⟨Ro.Th⟩</td>
<td>FH</td>
<td>88.3</td>
<td>9.4</td>
<td>10.6%</td>
<td>88.4</td>
<td>67.4</td>
<td>105.7</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>79.1</td>
<td>10.2</td>
<td>12.9%</td>
<td>78.4</td>
<td>53.1</td>
<td>100.5</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>93.3</td>
<td>9.1</td>
<td>9.7%</td>
<td>92.6</td>
<td>74.5</td>
<td>119.1</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>81.8</td>
<td>9.9</td>
<td>12.1%</td>
<td>83.0</td>
<td>58.9</td>
<td>107.2</td>
</tr>
<tr>
<td></td>
<td>L4</td>
<td>92.3</td>
<td>10.7</td>
<td>11.6%</td>
<td>92.0</td>
<td>64.5</td>
<td>115.2</td>
</tr>
<tr>
<td>⟨Pl.V⟩</td>
<td>FH</td>
<td>80.7</td>
<td>43.1</td>
<td>53.3%</td>
<td>68.4</td>
<td>12.4</td>
<td>243.4</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>69.7</td>
<td>55.3</td>
<td>79.3%</td>
<td>61.6</td>
<td>4.6</td>
<td>278.5</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>36.8</td>
<td>26.4</td>
<td>71.8%</td>
<td>29.8</td>
<td>9.8</td>
<td>145.6</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>20.5</td>
<td>13.4</td>
<td>65.7%</td>
<td>17.6</td>
<td>5.2</td>
<td>77.7</td>
</tr>
<tr>
<td></td>
<td>L4</td>
<td>32.8</td>
<td>21.8</td>
<td>66.5%</td>
<td>29.1</td>
<td>8.0</td>
<td>158.5</td>
</tr>
<tr>
<td>⟨Pl.S⟩</td>
<td>FH</td>
<td>1550</td>
<td>741</td>
<td>47.9%</td>
<td>1416</td>
<td>341</td>
<td>3937</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>1689</td>
<td>1242</td>
<td>73.5%</td>
<td>1468</td>
<td>175</td>
<td>5918</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>970</td>
<td>538</td>
<td>55.5%</td>
<td>822</td>
<td>362</td>
<td>3001</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>609</td>
<td>316</td>
<td>51.9%</td>
<td>527</td>
<td>205</td>
<td>1749</td>
</tr>
<tr>
<td></td>
<td>L4</td>
<td>849</td>
<td>410</td>
<td>48.3%</td>
<td>798</td>
<td>284</td>
<td>2559</td>
</tr>
<tr>
<td>⟨Pl.Th⟩</td>
<td>FH</td>
<td>81.0</td>
<td>6.5</td>
<td>8.0%</td>
<td>81.3</td>
<td>71.7</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>79.4</td>
<td>11.2</td>
<td>14.1%</td>
<td>81.0</td>
<td>59.6</td>
<td>108.4</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>94.7</td>
<td>9.1</td>
<td>9.6%</td>
<td>92.9</td>
<td>75.0</td>
<td>118.1</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>83.7</td>
<td>10.4</td>
<td>12.4%</td>
<td>83.7</td>
<td>63.7</td>
<td>111.8</td>
</tr>
<tr>
<td></td>
<td>L4</td>
<td>93.1</td>
<td>10.9</td>
<td>11.7%</td>
<td>91.3</td>
<td>68.6</td>
<td>118.1</td>
</tr>
</tbody>
</table>

| Pl.BV/BV | FH   | 83.0 | 10.3| 12.4% | 86.0 | 51.1 | 95.7  |
|          | IC   | 65.6 | 18.4| 28.0% | 69.3 | 6.0  | 88.0  |
|          | CA   | 33.8 | 15.7| 46.6% | 32.4 | 9.2  | 71.0  |
|          | L2   | 30.6 | 13.6| 44.6% | 29.4 | 7.3  | 61.4  |
|          | L4   | 34.9 | 14.2| 40.7% | 35.0 | 7.6  | 83.7  |

| El.BV_{\text{max}}/BV | FH   | 65.9 | 18.2| 27.7% | 71.3 | 16.2 | 87.7  |
|                       | IC   | 35.7 | 23.5| 65.8% | 32.2 | < 1.0| 78.4  |
|                       | CA   | 7.9  | 9.1 | 113.5%| 4.8  | < 1.0| 43.3  |
|                       | L2   | 6.0  | 6.4 | 107.9%| 3.8  | < 1.0| 39.2  |
|                       | L4   | 9.5  | 10.2| 107.3%| 7.0  | 1.2  | 69.8  |
Standard three-dimensional global morphometry was used to determine bone volume density (BV/TV) and the structure model index (SMI). These parameters have previously been reported and tabulated for the BIOMED I dataset [27] and were here used for linear regression analysis with the new local morphometric indices (Figures 2.6).

Results

The algorithm was first tested on three computer generated models. In all three cases the decomposition worked well and the result looked reasonable (Figure 2.3). Visual inspection confirmed that the algorithm basically should work on any structure type. The three parameters ($\langle \text{El.V} \rangle$, $\langle \text{El.S} \rangle$, $\langle \text{El.Th} \rangle$) computed by direct 3D morphometry were lying in the range of the analytically computed indices except for a few cases where the discrepancies between digital and analytical model were obvious (Table 2.2).

To investigate a realistic dataset we applied our algorithm to 328 cubic bone samples harvested from 70 donors at five different sites; femoral head (FH), iliac crest (IC), calcaneus (CA), and the second and fourth lumbar spine (L2, L4). The samples from the different sites varied dramatically in their architecture representing a unique dataset to test new algorithms. All structures were successfully decomposed by the proposed algorithm. Figure 2.4 shows the autopsy samples harvested from a 37-year-old male donor at the five different sites. These images show that most elements are separated in a very intuitive way. The L2 specimen represents a typically rod-like structure. In this structure the algorithm found many small rod-like elements building a loose trabecular network. Opposed to this the FH specimen represents a typically plate-like structure. Here we found one big element spanning through the whole specimen from top to bottom and from one side to the other (dark blue element). We would like to call these very large elements “major elements”. These major elements appear in trabecular bone structures that present themselves as a “Swiss cheese”, where only few nodes are present in the skeleton resulting in large elements spanning the whole structure.

To investigate the differences between sites in major elements we computed for each structure the volume of the largest element in percentage of the total bone volume ($\text{El.V}_{\text{max}}/\text{BV}$). Site specific distributions are shown in Figure 2.5A and Table 2.3. Some structures have one “strong” major element whereas others seem to be “weaker” structures composed of a loose network of mainly rod-like elements.

The structure type of the groups is also represented by the relative bone volume
Figure 2.5: Box plots of the locally determined indices for the five anatomical sites. (A) volume of the largest element in percent of the total bone volume, (B) relative amount of plate-volume in percentage of the total bone-volume. In (C) to (F), the distribution of the mean plate (C) and rod (D) volume, and plate (E) and rod (F) thickness as determined by local morphometry are shown.
fraction of plates (Pl.BV/BV). According to Figure 2.5B, and Table 2.3 structures of type CA, L2, and L4 may then be classified as typical rod-like structures, whereas the structures of type FH may be classified as typical plate-like structures. IC structures are typically a mix of plate- and rod-like structures yielding in a classification as an intermediate structure. Plate-like structures do not only have a higher relative plate-volume but the plates are also slightly larger (Table 2.3, Figure 2.5C). FH, and IC had significantly higher values then CA, L2, and L4 structures. Opposed to this, the size of the rods (Table 2.3, Figure 2.5D) could not be related to the structure type as determined by the relative amount of plate-volume. The values for FH and IC structures were lying in between the values of CA, L2, and L4.

The locally determined bone surface (⟨El.S⟩) demonstrates a similar pattern as the locally determined bone volume (⟨El.V⟩) for both, plates and rods (Table 2.3). This is not unexpected, since larger elements also have larger surfaces. Opposed to this, element thickness is independent of the trabecular size and does therefore not necessarily reflect ⟨El.V⟩ or ⟨El.S⟩ (Table 2.3, Figure 2.5E and 5F).

The structure model index (SMI), which in global morphometry is used to determine the rod-likeness or plate-likeness of a trabecular bone sample, was plotted

![Figure 2.6: Relationship of the globally determined structure model index (SMI) with the relative bone volume fraction of plates (Pl.BV/BV). A logarithmic fit (solid line) yields in a high correlation ($R^2 = 0.87; p < 0.001$). In the positive SMI range also a linear fit (dashed line) resulted in a good correlation ($R^2 = 0.81; p < 0.001$).](image-url)
2.1 Volumetric spatial decomposition of trabecular bone

versus the relative bone volume fraction of plates (Pl.BV/BV) (Figure 2.6). This relationship could be well described with a logarithmic law ($R^2 = 0.87$) for the whole dataset including negative SMI values and with a purely linear law ($R^2 = 0.81$) for the dataset where SMI was positive.

**Discussion**

In this paper we present a new method, that allows for the first time to spatially decompose trabecular bone and into its basic elements; plates and rods. This new method involves several consecutive algorithms that extract the elements from the sample, which then can be analyzed for size, shape and distributions using standard morphometric algorithms.

Spatial decomposition has been previously described based on line skeletons [20-22] which are not capable to include shape information and hence, cannot distinguish between plates and rods. A line skeleton is an excellent approach for topology analyses but when used for spatial decomposition the results might be difficult to interpret. Thus, these authors put their main interest in the topological analysis of the line skeletons and used the new parameters in combination with global morphometry [22]. Their results showed that local morphometric parameters might improve the prediction of mechanical properties of bones when combined with global morphometry. Another approach was done by analyzing shape preserved skeletons for orientation analysis of skeletonized trabeculae [26]. Their results showed to be a better measure of trabecular orientation than the classical mean intercept length (MIL) technique. Hence, they were able to show that it is important to analyze trabecular bone structures on an element level. The results from these studies corroborate the importance and the need of local morphometric analyses of trabecular bone structures, as presented in this paper. To our knowledge, this is the first study to analyze single trabecular rod and plate elements in their full volumetric extent.

To validate this new method, three computer generated models were created and then decomposed and analyzed by local morphometry. A rod-, mixed-, and plate-model were chosen to represent a typical range of possible structure types. Spatial decomposition followed by local morphometry of these models gave visually (Figure 2.3) and numerically (Table 2.2) good results.

Visually, the models were decomposed in intuitively correct elements (Figure 2.3). Consideration of the figures suggests that the algorithm basically should work on any structure type. Few limitations were found at the interfaces of the plates in the plate-model where the interconnecting horizontal plates were encompassed by
small wings intuitively belonging to the vertical plates. These artifacts arise from
the sharp angles as well as from the strict orientation of the plates along the image
axes. In realistic structures we do not expect such ideal configurations and thus,
these artifacts should not appear in real datasets. This could also be experimentally
validated, where in the analysis of the Biomed I data no such features were detected.

Numerically, the average element volume ($\langle \text{El.V} \rangle$), surface ($\langle \text{El.S} \rangle$), and thick-
ness ($\langle \text{El.Th} \rangle$) as computed by local morphometry were in good agreement to the
analytical derived solutions of corresponding models (Table 2.2). The values for
$\langle \text{El.V} \rangle$ and $\langle \text{El.S} \rangle$ all were relatively well centered within the analytic range. How-
ever, the already mentioned problem of the shape edges in the plate-model was also
reflected in the surface values of the plates, where the surface of the interconnecting
plates $P_C$ was slightly underestimated ($-17\%$) on the benefit of the vertical plates
$P_A$ (Figure 2.3). The $\langle \text{El.Th} \rangle$ values were all located close to the analytic ranges,
where the values for rods were on the lower, and the values for plates on the upper
end of the range. These artificial effects are due to the digital nature of the images
and are most pronounced in geometrically perfect objects. However, in true bone
there are no perfect rods or plates and it can be assumed that these effects average
out such that errors are about the same for all kind of elements.

To demonstrate that this new method does not only work on computer generated
models but also on real human trabecular bone samples, the algorithm was applied to
a set of 328 human vertebral bone samples harvested from 70 donors at five different
sites (femoral head: FH, iliac crest: IC, calcaneus: CA, second and fourth lumbar
spine: L2 and L4). This data has previously been described as part of the European
Union BIOMED I Concerted Action “Assessment of Bone Quality in Osteoporosis”
[27,45]. All samples could successfully be decomposed into its underlying volumetric
rods and plates. Visual inspection showed reasonable results for all structures and
sites. As an example structures from five anatomical sites of a 37-year-old male donor
are visualized in Figure 2.4. These images show rod-like structures for calcaneus
and the spine samples and a very dense plate-like structure in the femoral head.
The sample from iliac crest could either be classified as intermediate or plate-like
structure. Where in calcaneus and spine many small elements build up the structure
in the femoral head one big blue element is spanning through the whole sample.
Thus, already visual inspection reveals some site differences.

For numerical investigation, the spatially decomposed structures were analyzed
by local morphometry. Local morphometry in this sense means that standard global
morphometry algorithms were applied to each single element and that these results
were averaged for the whole structure independently for all rods and plates, respectively.

We hypothesize that the strength of strong, dense plate-like structures is determined by major elements whereas in spare, loosely connected rod-like structures the strength is given by the arrangement, quality and shape of a whole set of elements. Although we did not test bone strength in this project, identification of the largest element within each structure (Figure 5A) gives evidence for this hypothesis. In FH structures the strongest elements were in most cases at least half of the total bone volume. Thus, we think it is fair to suggest that these major elements carry most part of the load. In CA, L2, and L4 structures, we found major elements on the order of 5% of the total bone volume (Figure 2.5A). Such small elements may only be able to contribute locally to the competence of the specimen. In this case the arrangement, properties, and quality of the trabecular elements are much more important in determining bone strength. This statement is also supported by the fact that CA, L2, and L4 structures are mostly composed of rod-like elements (Figure 2.5B). In mechanical terms, plate-like structures are well suited to bear loads from one defined direction whereas rods-like structures show more flexibility as to the direction of the load. It is generally assumed that the choice which of the two structure types or hybrids thereof is available at a particular site is influenced by the “local” principal strain axis.

The plates from plate-like structures are generally larger than plates from rod-like structures (Table 2.3, Figure 2.5C) whereas the rods are of about the same size in all structures (Table 2.3, Figure 2.5D). First can be concluded by comparing Figure 2.5C, where the mean plate-size in FH and IC structures was significantly higher than in CA, L2, and L4 structures, with Figure 2.5B. Thus, plate-like structures do not only have more plate-volume but the plates are also larger. This could indicate that in a transformation from a plate-like structure to a rod-like structure plates are reduced in volume, perforated and finally may become a rod, as was suggested earlier [46]. On the other side, the ranges of the average rod-volume from CA, L2, and L4 structures enclose the ranges of the FH and IC structures (Table 2.3, Figure 2.5D). We therefore think that rods are of about the same size in all structures. We also found that the locally determined mean surface \( \langle \text{El.S} \rangle \) of the elements is proportional to the locally determined mean volume \( \langle \text{El.V} \rangle \) (Table 2.3). This is not unexpected, since larger elements also have a larger surface.

The data suggest that the thickness of trabecular elements is to some extent independent of structure and element type and therefore could be a biological constant. This hypothesis is supported by the results of the average thickness measurements.
for plates ($\langle\text{Pl.Th}\rangle$) and rods ($\langle\text{Ro.Th}\rangle$) as shown in Figure 2.5E and 5F. The range of thickness distribution is relatively narrow and the mean values are about the same for rods and plates (Table 2.3). This finding suggests that the thickness of trabeculae might be an invariant that is determined by biological regulation such as bone remodeling. Evidence for this hypothesis was provided in a study where human parathyroid hormone (PTH) was administered to cynomolgus monkeys [47]. These researchers found that PTH treatment increased cancellous bone volume, which was expressed on an architectural level by increased Tb.N and decreased Tb.Sp with no significant change in Tb.Th. They suggested that thickening of trabeculae may be an early event in response to PTH occurring prior to tunneling, which returns Tb.Th to normal levels while increasing Tb.N. However, this hypothesis could not be proven in our study and needs further biological investigations.

The definition of the structure type of a trabecular bone sample can be improved with local morphometry. This is due to the fact, that in local morphometry it is possible to decompose trabecular bone samples in rods and plates and hence, it is possible to compute the relative bone volume fraction of rods ($\text{Ro.BV/BV}$) and plates ($\text{Pl.BV/BV} = 100\% - \text{Ro.BV/BV}$). We propose to use this measure as a new parameter determining the structure type, where structures with less than 50% $\text{Pl.BV/BV}$ will be called rod-like structures and structures with more than 50% $\text{Pl.BV/BV}$ will be called plate-like structures. Conventionally, the structure model index (SMI) is used for an estimate of the rod-likeness and plate-likeness of a structure. This parameter was designed to yield values in the range from 0 for perfect plates to 3 for perfect rods [13]. SMI was used in many studies [14,48-53] to characterize trabecular bone architecture. In this study, we showed that the globally determined SMI and the locally determined $\text{Pl.BV/BV}$ were in good agreement when fitting a logarithmic law ($R^2 = 0.87$, Figure 2.6). For positive SMI values this logarithmic curve was almost linear resulting in a good linear fit for this range ($R^2 = 0.81$, Figure 2.6). However, for very dense, plate-like structures, SMI values become negative and thus exceed the definition range. Nevertheless, also these values are true measures and therefore need to be included in the analysis. This is due to the fact that SMI is only a derived parameter depending on the mean surface curvature $\langle H \rangle$, the bone volume $\text{BV}$ and the bone surface $\text{BS}$. The relation was shown to equal: $\text{SMI} = 12\langle H \rangle \text{BV/BS}$ [14]. Negative SMI values therefore reflect a negative mean curvature or so called “Swiss cheese” type structures. Opposed to this, $\text{Pl.BV/BV}$ cannot exceed its range which is defined from 0% (no rods) to 100% (no plates) and gives therefore an unambiguous classification of structure type. Furthermore, $\text{Pl.BV/BV}$ implicitly includes true structural information and provides a measure
for the relative amount of rods and plates in a trabecular structure. Opposed to this a structure with SMI = 1.5 may have a relative amount of plate volume that varies from 20% up to 70% (Figure 2.6).

In conclusion, we have developed a new method that allows for the first time a three-dimensional spatial decomposition of porous structures such as trabecular bone in its basic volumetric elements, i.e. plates and rods. The algorithm was validated on computer generated models where visual and numerical results were in good agreement with corresponding analytical models. Furthermore, 328 human trabecular bone samples from five different anatomical sites could spatially be decomposed and analyzed by local morphometry. We found that in plate-like structures major-elements span through the whole sample giving bone the main mechanical support, whereas in rod-like structures the mechanical support was determined by the quality, configuration and shape of a whole set of elements.

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References


2.1 Volumetric spatial decomposition of trabecular bone


2.1 Volumetric spatial decomposition of trabecular bone


2.2 A sensitivity analysis of the volumetric spatial decomposition algorithm

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Abstract:
Recently, we proposed a new method for the volumetric spatial decomposition of trabecular bone structures into its basic rod and plate elements. This method was based on a skeletonization approach, where two model parameters (Iterations and Length) were used to identify an ideal skeleton. The goal of this study was to estimate the sensitivity of local morphometric indices to the two model parameters. In a first step, the local morphometric indices of one sample were plotted as three-dimensional surfaces versus the two parameters. In a second step, twenty human trabecular bone samples were analyzed for intersections in linear plots. The results showed that the three-dimensional surfaces were smooth for rod derived indices ($R^2 > 0.993$) and had some randomness for plate derived indices ($R^2 > 0.910$). Furthermore, we found less intersections in the rod derived indices. For this reason, rod derived indices are more reliable than plate derived indices. The results also demonstrated that it was reasonable to reduce the model to one parameter by setting the parameter Length to twice the value of the parameter Iterations. In conclusion, we found that local morphometric indices are reliable measures that show large sample differences and thus may shed new light on structural differences of trabecular bone in a local fashion by adequately choosing one single optimization parameter.
Keywords:
volumetric spatial decomposition, sensitivity analysis

Introduction

Recently, we proposed a new method for the volumetric spatial decomposition of porous structures such as trabecular bone into its basic rod and plate elements [1]. This method was validated on computer generated models and showed reasonable results when applied to human trabecular bone specimens. The basic idea of this method was to reduce the volumetric object to a one-voxel thick homotopic skeleton containing only structural shape information. Applying a point-classification algorithm to the skeleton [2] allowed us to decompose the skeleton into one-voxel thick arcs and surfaces, which then could be expanded to their corresponding volumetric rods and plates by applying a dilation based algorithm.

The core of this method was thus to compute a reasonable skeleton serving for the decomposition. In our study, we used the term skeleton for an object that was derived from a 3D binary digital image and had the same topology and shape information as the original object but was only one voxel thick. Although this definition seems to determine the skeleton of an object pretty well, there are infinite ways one can think of on how to compute such a skeleton and many different approaches have already been proposed [3-11]. However, these algorithms perform usually well for the purpose they were designed for but may yield in only moderate results when applied to other applications. For this reason we used in our method a series of algorithms to compute the final skeleton.

In a first step, a computationally fast, isotropic, shape-preserving, and homotopic thinning algorithm [3,8] was used to compute a very rough two-voxel thick skeleton. This skeleton was then reduced to one-voxel thickness by a subsequent algorithm called conditional erosion [1]. The two algorithms were designed to maintain shape information such as plates and rods. Unfortunately, this means that also “imperfect rods” with an elliptical cross-section yield in a surface instead of an arc. To fix this problem, an optimization algorithm was used to reduce in a first step slender surfaces to arcs, and in a second step to remove end-arcs (an end-arc is an arc where one node is an arc end point) arising from object-surface noise. This optimization algorithm had two parameters which controlled 1) the number of iterations that had to be applied to transform thin slender surfaces into arcs and 2) the maximal length of end-arcs to be removed.

The goal of this study was to perform a sensitivity analysis for the two parameters
used in the optimization algorithm, and their effect on the skeleton, the decomposed structure and the derived morphometric indices. The first aim was to investigate whether the morphometric indices followed a smooth mathematical curve or whether they behaved chaotic in function of the optimization parameters.

Methods

Bone samples

Twenty human trabecular bone samples were extracted from intact spinal columns of nine donors who took part in an anatomical donation program. A specially developed drill was used to core the cylindrical specimens (10 mm height and 8 mm in diameter) from lumbar and thoracic vertebrae under constant water irrigation. Preceding drilling, contact radiographs were taken from each vertebra to orient them into a position such that the main trabecular orientation was aligned with the longitudinal axis of the cored specimen. No data was available on age and disease state of the donors. The samples were scanned using a micro-computed tomography system (\(\mu\)CT 40, Scanco Medical AG, Switzerland) at an isotropic nominal resolution of 20 \(\mu\)m. The reconstructed images were filtered using a constrained 3D Gaussian filter to partially suppress noise in the volumes (\(\sigma = 1.2\) voxel, support = 1 voxel), and binarized using a global threshold (12.4% of maximal possible gray scale value) as previously described [12]. A cylindrical region with a diameter of 7 mm was digitally cut, to exclude bone fragments that might have resulted from the cutting process. Component labeling was performed to remove unconnected parts of the structure.

Primary skeletonization

The component labeled images were then skeletonized using a three-dimensional homotopic, isotropic, and shape preserving thinning algorithm [3,8], yielding in a very rough two voxel thick, symmetric skeleton. Subsequently, a topology preserving algorithm called conditional erosion was used to reduce this skeleton to one voxel thickness (Figure 2.7B). A detailed description of these procedures is given elsewhere [1].
Skeleton optimization

The one-voxel thick skeletons resulting from primary skeletonization were a very rough representation of the original bone structure where rods with elliptic cross-section may be represented as slender surfaces. To overcome this problem, we applied the optimization algorithm as described in details below.

In a first step we used a point-classification algorithm that is able to compute

Figure 2.7: Optimization of the skeleton. A) very small subsection of a trabecular bone structure, B) primary skeleton, C) optimized with Iterations = 5, D) optimized with Iterations = 10.
2.2 A sensitivity analysis of the volumetric spatial decomposition for each voxel whether it is a surface point, a surface end point, an arc point, an arc end point, an arc-arc intersection point, an arc-surface intersection point, a surface-surface intersection point or an isolated point. The basic principle of this algorithm was introduced by Saha and Chaudhuri and is described in details elsewhere [2]. However, we had to modify this algorithm slightly since our skeletons were not ideal, which resulted in difficulties to detect certain intersection points.

To the classified skeletons we then applied two optimization procedures. The first was designed to reduce slender surfaces, which may appear from rod-like elements with elliptical cross-section, to arcs. To achieve this goal, we iteratively removed all surface-end points, which means that a one-voxel thick boundary is removed from all surfaces. After each iteration the skeleton was newly classified to retrieve a new properly classified skeleton (Figures 2.7C and 2.7D). If a surface was only two or three voxel thick prior an iteration the surfaces were turned into an arc. For the optimization we used a parameter which is simply the number of iterations that were applied to reduce the surfaces and hence will be called Iterations in this study.

The second algorithm was invented to remove end-arcs (an end-arc is an arc where one node is an arc end point) arising from object-surface noise. For this purpose, each rod which was shorter than a critical length was removed from the skeleton. The critical length defines a new parameter which in this study is called Length parameter.

Spatial decomposition

The optimized skeleton was spatially decomposed into rods and plates, where rods were identified to be the elements having exactly two nodes as opposed to plates that had more than two nodes. The elements were then expanded to their original size by applying a so called multi-color dilation algorithm [1]. This operation resulted in a spatially decomposed bone structure where all rods and plates were labeled with an individual color (or number).

Local morphometry

From this decomposed structure we computed local morphometric indices, which means that we applied standard three-dimensional morphometry to individual rods and plates. In order to follow the naming conventions proposed by Parfitt et al [13] we previously suggested to use the prefix Pl for plates, Ro for rods, and El for elements that could be either a plate or a rod. The volume, surface, and thickness of one single element were denoted with $V$, $S$, and $Th$, and the sum of the volume
and surface over all elements with BV, and BS, respectively. Furthermore, we use brackets \( ⟨⟩ \) to denote mean values that are averaged over all elements of the same type.

In this study we concentrated on the mean volume \( ⟨⟨Ro.V⟩⟩, ⟨⟨Pl.V⟩⟩ \), the mean surface \( ⟨⟨Ro.S⟩⟩, ⟨⟨Pl.S⟩⟩ \), as well as the mean thickness \( ⟨⟨Ro.Th⟩⟩, ⟨⟨Pl.Th⟩⟩ \) averaged for each structure over all rods and plates, separately. Additionally, the mean slenderness of rods \( ⟨⟨Ro.Sl⟩⟩ \) was computed as the ratio of the rod length to thickness, where the length was the node distance in the corresponding skeletonized image. Furthermore, the relative bone volume fraction of plates \( Pl.BV/BV \) and rods \( Ro.BV/BV = 100% - Pl.BV/BV \) in percentage, as well as the number of rods \( Nr.Rods \) and plates \( Nr.Plates \) were determined.

**Sensitivity analysis: Iterations and Length for one sample**

For the sensitivity analysis of the two parameters Iterations and Length, an arbitrary sample was selected and optimized using a series of Iterations and Length combinations. Both parameters were varied in the range of 0 to 48 in steps of 3. For each optimized skeleton local morphometry was computed.

To investigate the influence of the Iterations parameter in more details, we plotted the data points at constant \( Length = 30 \) versus Iterations and fitted a Gompertz relation or rational function, whatever gave better results. These two mathematical functions showed to yield in reasonable fits for all parameters:

\[
\begin{align*}
  f(x) &= ae^{-b-cx} \\
  f(x) &= \frac{a + bx}{1 + cx + dx^2}
\end{align*}
\]

Gompertz Relation  
Rational Function

**Sensitivity analysis: Intersection of independent samples**

To investigate whether independent samples were similarly affected by the optimization procedure, a set of twenty samples was optimized using the parameter sets \( (Iterations/Length) = \{(3/6), (6/12), (9/18), (15/30), (21/42), (30/60), (48/96)\} \), where we chose multiples of three to be consistent with the one-sample analysis.
2.2 A sensitivity analysis of the volumetric spatial decomposition

Figure 2.8: Three-dimensional surface plots to explore the sensitivity of the morphometric indices in function of the two model parameters Iterations and Length.
Figure 2.9: Three-dimensional surface plots to explore the sensitivity of the morphometric indices in function of the two model parameters Iterations and Length.
Results

Sensitivity analysis: Iterations and Length for one sample

To investigate the effect of the two optimization parameters Iterations and Length, the basic morphometric indices were displayed as three-dimensional surface plots for one sample (Figures 2.8 and 2.9). Generally, all these surfaces were much more sensitive to Iterations than to Length. The later had only an effect up to a value of 30, where the effects were most pronounced in the indices Nr.Rods, ⟨Ro.Sl⟩, ⟨Ro.V⟩, and ⟨Ro.S⟩. Thus, if for Length a sufficiently high value is chosen the optimization procedure can be simplified to a one parameter algorithm.

To analyze the three-dimensional surface plots in more details with respect to Iterations, one-dimensional plots along the isoline Length = 30 were created (Figure 2.10). To all these plots a Gompertz relation (Figures 2.10A, 2.10B, and 2.10H) or rational function (Figures 2.10C – 2.10G, and 2.10I – 2.10K) could be fitted that accurately described the characteristic of the curve. An exception was made for Nr.Plates, where the first data-point was excluded prior fitting the curve. In Figures 2.10A – 2.10H the curves fitted very well with $R^2 > 0.993$ and in Figures 2.10I – 2.10K the curves fitted also well with $R^2 > 0.910$.

The relative bone volume fraction of rods (Ro.BV/BV), which can be used as a measure of the plate/rod characteristic of a trabecular bone sample (previous section), strongly depends on the parameter Iterations (Figures 2.8A and 2.10A). In the sample analyzed Ro.BV/BV changed its structure type from a plate-structure to a rod-structure, where the sigmoidal increase was largest and almost linear in the range of 3 to 30. The relative bone volume fraction of plates (Pl.BV/BV) showed the inverse behavior (Figures 2.8B and 2.10B).

The number of rods (Nr.Rods) increased monotonically, where the increase was largest for small Iterations (Figures 2.8C and 2.10C). This is consistent with an increase in Ro.BV/BV. The number of plates showed a discontinuity at the first step followed by a decent decrease that could accurately be modeled using a rational function (Figures 2.8D and 2.10D). To avoid this discontinuity the parameter Iterations should therefore not be set to zero.

The mean indices derived from the rods (⟨Ro.Sl⟩, ⟨Ro.V⟩, ⟨Ro.S⟩, ⟨Ro.Th⟩) all showed a similar increase that was relatively steep for low Iterations and started to level out at higher values. The increase was most pronounced in ⟨Ro.Th⟩, which reached its level already around 10 (Figures 2.9E and 2.10H), followed by ⟨Ro.Sl⟩, which reached its level at about 20 (Figures 2.8E and 2.10E). The indices ⟨Ro.V⟩
(Figures 2.9A and 2.10F) and $\langle \text{Ro.S} \rangle$ (Figures 2.9C and 2.10G) both showed an increase that was similar to Nr.Rods.

The mean indices derived from the plates ($\langle \text{Pl.V} \rangle$, $\langle \text{Pl.S} \rangle$, $\langle \text{Pl.Th} \rangle$) could not be perfectly modeled by using a reasonable mathematical function and showed some random behavior. The deviation from the data points to the fitted curve was almost 10% which makes these indices less reliable. Similarly to Nr.Plates, the first two data-points had to be omitted indicating that small values for Iterations should be

Figure 2.10: These plots show sections through the three-dimensional surfaces plotted in Figure 2.8 and 2.9 for a constant Length = 30.2 and 3 for a constant Length = 30.
avoided. Apart from these points, the indices $\langle \text{Pl.V} \rangle$ (Figures 2.9B and 2.10I) and $\langle \text{Pl.S} \rangle$ (Figures 2.9D and 2.10J) showed a similar decrease with increasing Iterations, and the values of $\langle \text{Pl.Th} \rangle$ (Figures 2.9F and 2.10K) were slightly decreasing at higher Iterations.

Figure 2.11: Intersection plots for twenty independent samples.
Sensitivity analysis: Intersections of independent samples

To investigate the relative sensitivity of morphometric indices on the parameters Iterations and Length in different trabecular bone samples, we applied the optimization algorithm to twenty bone samples and displayed the results as intersection-
2.2 A sensitivity analysis of the volumetric spatial decomposition plots (Figures 2.11 and 2.12). To simplify the optimization algorithm the parameter Length was set to twice the parameter Iterations, which was shown to be a reasonable setting based on visual inspection of the data.

In Ro.BV/BV (Figure 2.11A) and Pl.BV/BV (Figure 2.11B) we found the samples to behave similarly and the lines mostly showed no intersections. The variation between the samples was largest in the range for Iterations from 3 to 30. Thus, this range would be ideal to find sample differences according to Ro.BV/BV or Pl.BV/BV, which showed to be reliable indices.

In Nr.Rods (Figure 2.11C) most lines had a parallel course, which makes this index reliable. However, a few samples leveled out already at low values for Iterations and thus intersected the lines of other samples. These intersections were especially pronounced in the range up to about 30. Opposed to this, the samples showed many intersections in Nr.Plates (Figure 2.11D), which were especially pronounced for low values of Iterations. Above 30 these intersections mostly vanished.

The mean indices derived from the rods ($\langle \text{Ro.nm} \rangle$, $\langle \text{Ro.V} \rangle$, $\langle \text{Ro.S} \rangle$, $\langle \text{Ro.Th} \rangle$) showed all only moderate intersections that could be neglected for values of Iterations larger than 20. Above this range, these indices were mostly constant, wherefore for these indices, a value above 20 would yield in reasonable results (Figures 2.11E, 2.12A, 2.12C, 2.12E).

The mean indices derived from the plates ($\langle \text{Pl.V} \rangle$, $\langle \text{Pl.S} \rangle$, $\langle \text{Pl.Th} \rangle$) showed many intersections over the whole range (Figures 2.12B, 2.12D, 2.12F). Additionally, the curves did not follow simple mathematical functions.

Discussion

In this study, we analyzed the sensitivity of the volumetric spatial decomposition method on its two model parameters Iterations and Length. The sensitivity was explored by computing the basic local morphometric indices for different optimization schemes. In a first step, a single sample was analyzed to find independent effects of these two parameters. Since we found that Length had only minor effects on the outcomes, further analyses concentrated on the parameter Iterations. In a second step, twenty samples were analyzed to find whether the influence of the model parameters acted similarly on different samples.

Generally, the morphometric indices were much more dependent on the optimization parameter Iterations than on the parameter Length. This is not unexpected; the Length determines the cut-off length for free-ending skeleton arcs to be removed.
from the skeleton. At some point all free-ending arcs are removed and thus the skeleton does not change anymore if this parameter is increased. This optimization parameter has therefore only a minor importance and the three-dimensional surface plots (Figures 2.8 and 2.9) suggest, that for sufficiently high values of Length the outcome is invariant in this parameter and thus the optimization procedure can be reduced to a one parameter model. We suggest setting the parameter Length to twice the value of the parameter Iterations, which should yield in sufficiently high values in all cases. For this reason, further discussions concentrate to the discussion of the Iterations parameter.

The relative bone volume fraction of rods (Ro.BV/BV), and with that the derived structure type, strongly depended on the setting of the Iterations parameter (Figures 2.8A and 2.10A). If this parameter becomes sufficiently high eventually any structure may turn into a complete rod-structure. This is intuitively clear, since at some point all surface-edge points in a classified skeleton may be removed and thus plates are turned into a set of rods. For this reason, the Iterations parameter should not be chosen too high. The intersection plots revealed that the variation in Ro.BV/BV was largest in the range from 3 up to about 30. Thus, any parameter in this range should yield in reasonable results concerning the rod/plate characteristic of the structures as well as concerning sample differences. The same is true for Pl.BV/BV (Figures 2.8B and 2.10B).

Closely related to this is the number of rods (Nr.Rods), which also increased with increasing Iterations (Figures 2.8C and 2.10C), a result caused by the reduction of surfaces to arcs. Here, the Length parameter had a more pronounced effect which clearly indicated that a big part of noise could be removed by setting this parameter. The intersection plots (Figure 2.11C) showed that not all trabecular bone structures behaved the same as a function of Iterations. If a structure is extremely rod-like only few plates may be converted to rods wherefore Nr.Rods levels out already at low Iterations. Nevertheless, there were only a few lines that showed intersections and most lines were on a parallel course. The intersections could even be neglected after the value of 30. Opposed to this, the number of plates (Nr.Plates) has to be considered with more care. In the one sample plot (Figure 2.10D) we found a discontinuity at the first step followed by a smooth decrease that could be modeled well using a rational function. However, the corresponding intersection plot (Figure 2.11D) demonstrates that this index is difficult to interpret due to the many intersections which were especially pronounced for low Iterations. Above 30 these intersections mostly vanished. However, the variance was also seriously reduced in this range, wherefore not many sample differences can be expected. Although, there
is a tendency that in all samples Nr.Plates decreases, comparison of samples in this index should be taken with care.

The morphometric indices averaged for all rods could accurately be modeled by a mathematical law (Figures 2.10E – 2.10H) and showed only minor intersections (Figures 2.11E, 2.12A, 2.12C, and 2.12E). The mean slenderness (⟨Ro.Sl⟩) and the mean thickness (⟨Ro.Th⟩) of the rods showed a steep increase for low values of Iterations and were basically constant for values above 20. For these two indices a high Iterations value leads in a reproducible result. The mean volume (⟨Ro.V⟩) and the mean surface (⟨Ro.S⟩) of the rods showed a similar course where the initial increase was less steep. For this reason it is no problem to compare different bone structures in these indices, where differences become more pronounced at higher Iterations.

The morphometric indices averaged for all plates showed some randomness, wherefore interpretations based on these indices must be taken with care. Especially ⟨Pl.Th⟩ showed high intersections for larger values of Iterations (Figure 2.12F). However, for values below 20, a reasonable estimation of plate thickness should be possible, since in this range the variation was moderate. The mean volume (⟨Pl.V⟩) and the mean surface (⟨Pl.S⟩) generally decreased with increasing Iterations (Figures 2.12D and 2.12F). Although some samples behaved chaotic, there were only moderate intersections. Since no perfect mathematical model could be fitted (Figures 2.10I – 2.10K), the scaling of morphometric indices with respect to Iterations is only possible with limited accuracy.

These specific results demonstrate that the outcome of local morphometric indices depend on the Iterations parameter, wherefore this parameter has to be chosen carefully and should be fixed for each study. An optimal parameter for the present study lies in the range of 20 to 30. In this range the results are scalable with high precision for indices derived from rods and with moderate precision for indices derived from plates. However, the exact parameter setting is also resolution dependent, wherefore this result can not be generalized and is only valid for a nominal resolution of 20 µm as used in this study. If a different resolution is chosen, the parameter has to be determined once again, either, by visual inspection or by a similar sensitivity study. Furthermore, it has to be noted that the specimens in this study were all harvested from human lumbar spine and were thus rather rod-like. In specimens from different sites with more plate structures, the sensitivity could behave differently and an other parameter could become optimal.

In conclusion, we found that the optimization algorithm of the volumetric spatial decomposition algorithm can be reduced to a one parameter model, by setting the
Length parameter to twice the value of the Iterations parameter. If later parameter is chosen well, local morphometric indices are reliable measures that show large sample differences and thus may shed new light on structural differences of trabecular bone in a local fashion.

Acknowledgement

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References


2.2 A sensitivity analysis of the volumetric spatial decomposition


Chapter 2. Volumetric spatial decomposition
Chapter 3

Local morphometry
3.1 Age-related changes in trabecular bone microstructures: global and local morphometry

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Abstract:
A recently developed method allows investigating trabecular bone on an elemental (rod/plate) level. With this method, it is possible to measure local morphometric parameters such as thickness or orientation directly on the extracted rods and plates. Age-related changes of trabecular microarchitecture can thus be investigated on an elemental level, which may help to improve the understanding of age-related bone failure mechanism as well as the effect of pharmaceutical intervention in the prevention of such fractures.

Autopsies from femoral heads (FH), iliac crest (IC), and lumbar spine (LS) were analyzed by global morphometry. Additionally, the trabecular structures were decomposed into rods and plates for the analysis with local morphometry. These morphometric indices were related to age using an analysis of covariance to test for gender differences and linearity with age.

In this study, age-related changes showed no gender but site differences. In LS, rods were thinned in aging and finally vanish from the structure, causing a transformation of the trabecular bone structure to longer and, on average, thicker rods. In IC we found a high variation in the morphometric indices at all ages, thus it was difficult to formulate a general behavior for this site. In FH, changes were expressed by a simultaneous thinning and loss of interconnecting trabeculae and perforation of plates leading to new plates and rods. Results were mostly in agreement with earlier findings mostly using descriptive analysis of the aging process. Here we present for the first time preliminary quantitative evidence of
changes in the local microstructure, i.e., individual rods and plates. Nevertheless, the number of samples was too small to make for ready conclusions. We conclude that the combination of local and global morphometry is a useful method for a detailed and quantitative description of age-related changes in bone microstructure.

**Keywords:**
age-related changes, local morphometry, osteoporosis, trabecular bone, trabecular plates, trabecular rods

**Introduction**

Age and disease related osteoporotic bone fractures are now considered a major burden to society in most developed countries. The total medical expenditures of osteoporotic hip-fractures, which is related with the highest medical costs, has been estimated for many countries [1-9]. On a global scale, it was estimated that the number of hip fractures occurring in the world each year will rise from 1.66 million in 1990 to 6.26 million by 2050 [10]. Thus, assuming a total annual cost of roughly US$ 30'000 per hip-fracture case [8] the global expenditures will rise from US$ 50 billion in 1990 to almost US$ 200 billion in 2050.

The definition of osteoporosis changed over the years with increasing knowledge over this disease. Where in the earlier days osteoporosis was defined as an absolute decrease in the amount of bone [11-13] to a level below that required for mechanical support [14], later definitions added the importance of microarchitecture [15,16] and bone quality [17], a term referring to architecture, turnover, damage accumulation and mineralization. With the definition of osteoporosis also the focus of investigation changed over the years. Most early studies in age-related bone changes concentrated in the description of the amount of bone volume [11-13,18-27] and only few investigated structural parameters such as bone surface [21,24,25], trabecular number [24,28], trabecular thickness [22,24], or trabecular spacing [22]. This was based on the intuitive idea that the mechanical properties of trabecular bone depend strongly on its apparent density or bone volume [29,30] and it became an indisputable fact that bone is lost in aging people. However, Hui *et al* demonstrated that even for constant bone mass, fracture risk increases with age [31]. Thus, bone quality changes with age independent of bone mass and components such as microarchitecture, turnover, damage accumulation and mineralization must be taken into account in determining bone strength.
Parfitt et al were first to highlight the evidence of trabecular bone microstructure in the etiology of osteoporosis and bone fractures [32]. With their newly introduced parallel-plate model they investigated architectural changes with age such as spacing and thickness of trabecular plate structures and found that the reduction in bone volume was mainly due to a reduction in plate density, with no significant decrease in plate thickness [32,33]. The authors proposed that the process of plate-removal was initiated by an excessive depth of osteoclastic resorption cavities, leading to focal perforation of plates, followed by progressive enlargement of the perforation with conversion of plates to rods. It was also proposed that a potential thinning of residual elements could arise from incomplete refilling by osteoblasts of resorption cavities of normal or reduced size [34]. Additionally, it was speculated that vertical elements that are subjected to compression could increase in thickness as a compensatory response to loss of horizontal trabeculae [35]. These results corroborated the theory that not only bone density but also structural parameters play a leading role in the determination of trabecular bone competence, and it was also recognized that fenestration and loss of connectivity can probably only partially be repaired by adding bone to existing surfaces [36,37].

This theory stimulated research in trabecular bone structure related to aging and different methods were used to investigate the thickness and spacing of trabecular elements. Mosekilde used polarized light to demonstrate for human vertebrae that the mean horizontal trabecular thickness was significantly decreased with age whereas the mean thickness of vertical trabeculae remained unchanged with age [38]. Using the same technique she found that the decrease in trabecular bone volume density, ash-density, and mean horizontal trabecular thickness and the increase in trabecular separation were identical for males and females [39]. Similarly, Thomsen et al found in their histological sections using a modified version of the parallel plate model that, the vertical trabecular thickness was independent of age, whereas the thickness of horizontal trabeculae decreased significantly with age [40]. The trabecular number and trabecular spacing was found to decrease and increase, respectively for both, vertical and horizontal trabeculae. Bergot et al found by using granulometry, that the mean width of vertical trabeculae decreased in both men and women with age, but that the mean width of horizontal trabeculae decreased with age only in women [41]. From this, they concluded that age-related loss of trabecular bone can be attributed to at least two processes: generalized trabecular thinning of trabeculae as well as shortening or complete disappearance of some trabeculae. With histomorphometric analysis McCalden et al showed that the surface to volume ratio and the mean separation of the trabecular plate increased with age, whereas the mean thick-
ness and connectivity of the trabecular plate decreased [42]. Histomorphometry was also used by Weinstein et al who concluded that the decrease in trabecular bone with advancing age is due to both an increase in the distance between trabeculae and a decrease in the width of individual trabecular profiles [43].

With the improvement in spatial resolution of three-dimensional imaging systems such as micro-computed tomography (µCT) [44], peripheral quantitative-computed tomography (QCT) [45,46], and magnetic resonance imaging (MRI) [47-49] it became possible to assess trabecular bone microstructure in three-dimensional space. This pushed the research field towards the assessment of new unbiased three-dimensional quantities such as the model-independent assessment of mean trabecular thickness (Tb.Th) and mean trabecular separation (Tb.Sp) [50], structure model type (SMI) [51], mean curvature of trabecular bone structure (⟨H⟩) [52], or connectivity density (Conn.D) [53]. All these parameters were also related to changes with age and diseases. Ding et al found that SMI changed towards more rod-like structures in the elderly, and that Tb.Th declined significantly with age [54]. These changes became significant after the age of 80 years and remained relatively unchanged between 20 and 80 years. They also revealed an age-related increase in architectural anisotropy (DA), age-related decrease in bone volume fraction (BV/TV) and bone surface density (BS/BV), and age-independence of connectivity in the condyle of the tibia [55]. Their results suggest that bone remodeling in aging tibial cancellous bone may reorient trabecular volume orientation rather than selectively removing trabecular struts oriented in a particular direction. These findings are in line with finite element model simulations [56,57] demonstrating the adaptation of bone structure orientation along the principal loading axis by simulating cellular mechanism. Another study highlighted, that three-dimensional connectivity measurements are not able to discriminate between rod-like connections and fenestrated plates and hence should not be used as an age-related measure [58].

Recently, we proposed a new method for the local morphometric analysis of single trabecular rod and plate elements [59-61]. This new method allows computing morphometric parameters such as volume, surface, thickness and orientation directly on a trabecular level (i.e. individual rods and plates) and it is for the first time possible to verify in three-dimensional space whether the thickness of horizontal and vertical rods are age-dependent. Local morphometry also opens the doors to investigate new parameters such as the slenderness of rods, which is important in determining whether buckling is a potential failure mode. To complete the picture in age-related changes of trabecular bone we also included global morphometric parameters such as BV/TV and Tb.Sp, which are better measured by means of
global than local methods. The overall aim of this study was to achieve a better understanding in age-related changes of trabecular bone.

Methods

In this study, we analyzed human trabecular bone samples assessed as part of the European Union BIOMED I Concerted Action “Assessment of Bone Quality in Osteoporosis” [62,63]. The analyzed dataset encompasses 182 samples harvested from 70 donors at three different anatomical sites; femoral head (FH), iliac crest (IC), and second lumbar spine (LS). Bone samples were recruited from subjects in the postmortem room in standardized conditions from subjects of all ages and different pathological conditions. The main causes of death were heart failure, pneumonia, stroke, and sepsis. The samples were extracted along their site-specific anatomical loading axis. Detailed clinical characteristics of the patients included in this BIOMED I project have been described elsewhere [64,65]. No patients were excluded due to known skeletal diseases, metabolic diseases nor to drugs known to affect the skeleton. In that sense, the study population was not considered a normal population with respect to their bone status but a typical cross-section of the hospital population at the time of the study. The numbers of samples and mean ages per site and sex are shown in Table 3.1.

All samples were scanned using a micro-computed tomography (µCT) system (µCT 20, Scanco Medical AG, Switzerland) providing a spatial resolution of 28 µm. This system and scanning procedure is described elsewhere in detail [44] and the dataset has previously been used for several investigations [61,62,66]. A 4-mm cubic region of interest (TV) was selected from all samples. Using Gaussian filtration and global thresholding [62], binary images were created presenting either bone or background. A component labeling algorithm was applied to the binary images to remove all parts not connected to the main structure.

These images were used for both, global and local morphometry. For global morphometry, standard 3D algorithms were used to compute bone volume density (BV/TV), bone surface (BS/TV), and bone surface density (BS/BV), as well as trabecular spacing (Tb.Sp), trabecular number (Tb.N), and trabecular thickness (Tb.Th). These algorithms are described in details elsewhere [50,62]. Furthermore, we also computed the mean surface curvature (⟨H⟩) [52] also denoted to as trabecular bone pattern factor (TBPF) [67], and the structure model index (SMI) [51]. All these parameters were computed in three-dimensional space without using any model assumptions and are summarized in Figure 3.2 and Table 3.2.
For local morphometry, the component-labeled images were first spatially decomposed into rods and plates. A detailed description of the volumetric spatial decomposition algorithm as well as local morphometry can be found elsewhere [61] where here only a short description of the main procedures is provided. First, the component labeled images were skeletonized using a three-dimensional homotopic, isotropic, and shape preserving thinning algorithm [68,69], yielding in a two-voxel thick, skeleton, invariant with respect to any axis permutation. Subsequently, a topology preserving algorithm called conditional erosion was used to reduce this skeleton to one voxel thickness [61]. These skeletons are a very rough, one voxel thick representation of the original bone structure where rods with elliptic cross-section may be represented as slender plates. To overcome this problem, we applied an optimization algorithm to the skeleton to get a reasonable representation of rods and plates and to reduce surface noise. The slenderness-parameter used for this optimization step was set to ten, which was visually yielding in reasonable results [61]. The optimized skeleton was then characterized by a slightly modified point-classification algorithm originally devised by Saha et al [70], which was able to compute for each voxel within the skeleton whether it was a surface point, a surface end point, an arc point, an arc end point, an arc-arc intersection point, an arc-surface intersection point, a surface-surface intersection point or an isolated point. This classified skeleton was spatially decomposed into rods and plates, which were expanded to their original size by applying a so called multi-color dilation algorithm [61]. This operation resulted in a spatially decomposed trabecular bone structure where all rods and plates were labeled with an individual number (Figure 3.1A and 3.1C).

Table 3.1: Number and age-ranges of the trabecular bone samples per gender and site.

<table>
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<tr>
<th>site</th>
<th>female</th>
<th></th>
<th></th>
<th>male</th>
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<td></td>
<td></td>
<td>FH</td>
<td>IC</td>
<td>LS</td>
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<td>IC</td>
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<tr>
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<td>26</td>
<td>26</td>
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<tr>
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<td>0.06</td>
<td>0.16</td>
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</tbody>
</table>

*site-wise students t-test for female vs. male.
3.1 Age-related changes in trabecular bone microstructures

Figure 3.1: Spatial decomposition of trabecular bone structures into rods and plates. The images on the left-hand side show the extracted elements after the spatial decomposition by different colors, the images on the right-hand side show which elements were identified as rods (blue) and plates (red), respectively. The three structures are from femoral head (A, B), iliac crest (C, D), and lumbar spine (E, F) to represent a plate-like, hybrid and rod-like trabecular bone structure.
Local morphometry was then used to compute element based indices [61]. These indices were computed and averaged for rods and plates separately (Figure 3.1B and 3.1D). The length of the rods (⟨Ro.Le⟩) was determined directly from the point-classified skeleton by computing the distance of the two nodes. This operation was applied only to rods, since the orientation of plates is not clearly defined. The orientation of the rods (⟨Ro.θ⟩) was defined as the angle spanned by the line connecting the two nodes and the z-axis of the image. Since all samples were extracted along their site-specific loading axis, the z-axis was collinear with the loading axis, and thus, the orientation represents the orientation as compared to the loading axis. We used this angle to group vertical elements (θ ≤ 45°) and horizontal elements (θ > 45°). All other parameters such as the thickness or volume of the elements were determined by applying standard morphometry algorithms to the rods and plates. In this paper we denote ⟨Ro.Th⟩ and ⟨Pl.Th⟩ to be the mean thickness of all rods and plates respectively in a structure. The mean thickness of all vertical and horizontal rods are denoted to as ⟨RoV.Th⟩ and ⟨RoH.Th⟩, respectively. The mean slenderness of the rods, as computed from the length over the thickness, is denoted to ⟨Ro.Sl⟩. Furthermore, we computed the plate volume density (Pl.BV/BV), which is defined as total plate volume divided by total volume of interest in percent. This parameter was shown to represent structure type similar to the globally determined SMI but in a more direct way [61].

For the statistical analysis, the GNU statistical computation and graphics package R (Version 2.0.1; http://www.r-project.org) was used. We conducted an analysis of covariance to determine whether the morphometric indices had a linear trend with age and to find differences between women and men. For the indices that showed an age-related linear trend we used the linear model to express these changes. The linear regression was expressed by arbitrarily setting the value at 30 years (young adult) to 100%, which allowed us to express the increase/decrease of a certain parameter in percentage per decade, relative to this age. With this, the regression line is clearly defined and the 100% level, which was arbitrarily chosen at the age of 30, could easily be moved to any other age. This analysis of covariance was performed site wise and the resulting p-values are shown in Table 3.2 and Table 3.3 for global and local morphometry, respectively. The data was visualized by scatter plots and lowess fits [71,72] were used to highlight trends (Figures 3.2 – 3.4). The same statistical analysis was performed for all samples of donors aged 59 years and older.
Results

Generally, we can say that for all morphometric indices the variation between subjects in any given site was large as compared with age-related changes. The variation was especially large in the iliac crest samples. For this reason, it was difficult to formulate general laws and for most parameters it was not so clear whether the relation with age was linear. Nevertheless, the data gives important indications and trends in bone changes with increasing age. The levels and trends of most morphometric indices were different for the three sites.

Table 3.2: P-values of the analysis of covariance for the global morphometric indices.

<table>
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<th>Site</th>
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<th>gender</th>
<th>age:gender‡</th>
<th>change†</th>
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<td>0.132</td>
<td>0.770</td>
<td>–</td>
</tr>
<tr>
<td></td>
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<td>0.556</td>
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<tr>
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<td>0.897</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>0.081</td>
<td>0.267</td>
<td>0.377</td>
<td>–</td>
</tr>
<tr>
<td></td>
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<td>&lt;0.001</td>
<td>0.356</td>
<td>0.399</td>
<td>-6.8</td>
</tr>
<tr>
<td>BS/BV</td>
<td>FH</td>
<td>0.112</td>
<td>0.263</td>
<td>0.533</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>0.923</td>
<td>0.528</td>
<td>0.063</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>0.128</td>
<td>0.743</td>
<td>0.494</td>
<td>–</td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>FH</td>
<td>0.157</td>
<td>0.329</td>
<td>0.779</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
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<td>0.246</td>
<td>0.485</td>
<td>6.2</td>
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<tr>
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<td>0.506</td>
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<tr>
<td>Tb.N</td>
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<td>0.123</td>
<td>0.177</td>
<td>0.896</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>0.006</td>
<td>0.370</td>
<td>0.531</td>
<td>-5.2</td>
</tr>
<tr>
<td></td>
<td>LS</td>
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<td>0.656</td>
<td>0.856</td>
<td>-3.6</td>
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<tr>
<td>Tb.Th</td>
<td>FH</td>
<td>0.201</td>
<td>0.531</td>
<td>0.584</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
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<td>0.619</td>
<td>0.101</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>0.480</td>
<td>0.637</td>
<td>0.896</td>
<td>–</td>
</tr>
<tr>
<td>⟨H⟩</td>
<td>FH</td>
<td>0.077</td>
<td>0.052</td>
<td>0.837</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>0.719</td>
<td>0.828</td>
<td>0.162</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>0.463</td>
<td>0.668</td>
<td>0.229</td>
<td>–</td>
</tr>
<tr>
<td>SMI</td>
<td>FH</td>
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<td>0.070</td>
<td>0.528</td>
<td>–</td>
</tr>
<tr>
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<td>0.241</td>
<td>0.589</td>
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</tr>
<tr>
<td></td>
<td>LS</td>
<td>0.012</td>
<td>0.268</td>
<td>0.357</td>
<td>6.0</td>
</tr>
</tbody>
</table>

*interaction term age:gender.
†change per decade in percent.
‡bold numbers are smaller than 0.05.
Table 3.3: P-values of the analysis of covariance for the local morphometric indices.

<table>
<thead>
<tr>
<th>Index</th>
<th>Site</th>
<th>age</th>
<th>gender</th>
<th>age:gender</th>
<th>change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr.Rods</td>
<td>FH</td>
<td>0.469</td>
<td>0.356</td>
<td>0.952</td>
<td>–</td>
</tr>
<tr>
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<td>IC</td>
<td>0.358</td>
<td>0.253</td>
<td>0.675</td>
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</tr>
<tr>
<td></td>
<td>LS</td>
<td><strong>0.002</strong></td>
<td>0.626</td>
<td>0.788</td>
<td>-7.9</td>
</tr>
<tr>
<td>Nr.Rods/Nr.Plates</td>
<td>FH</td>
<td>0.093</td>
<td>0.174</td>
<td>0.916</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>0.624</td>
<td>0.250</td>
<td><strong>0.049</strong></td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>0.235</td>
<td>0.786</td>
<td>0.072</td>
<td>–</td>
</tr>
<tr>
<td>Pl.BV/BV</td>
<td>FH</td>
<td>0.155</td>
<td>0.104</td>
<td>0.991</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>0.821</td>
<td>0.851</td>
<td>0.154</td>
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</tr>
<tr>
<td></td>
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<td>0.775</td>
<td>0.318</td>
<td>0.379</td>
<td>–</td>
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<tr>
<td>⟨Ro.Sl⟩</td>
<td>FH</td>
<td>0.019</td>
<td>0.445</td>
<td>0.455</td>
<td>4.2</td>
</tr>
<tr>
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<td>0.777</td>
<td>0.301</td>
<td>0.123</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td><strong>0.019</strong></td>
<td>0.336</td>
<td>0.987</td>
<td>2.7</td>
</tr>
<tr>
<td>⟨Ro.Le⟩</td>
<td>FH</td>
<td>0.151</td>
<td>0.464</td>
<td>0.719</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>0.666</td>
<td>0.381</td>
<td>0.432</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>&lt; <strong>0.001</strong></td>
<td>0.122</td>
<td>0.973</td>
<td>5.7</td>
</tr>
<tr>
<td>⟨Ro.Th⟩</td>
<td>FH</td>
<td>0.384</td>
<td>0.562</td>
<td>0.199</td>
<td>–</td>
</tr>
<tr>
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<td>0.647</td>
<td>0.658</td>
<td>0.847</td>
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<tr>
<td></td>
<td>LS</td>
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<td>0.147</td>
<td>0.894</td>
<td>2.6</td>
</tr>
<tr>
<td>⟨Pl.Th⟩</td>
<td>FH</td>
<td>0.539</td>
<td>0.170</td>
<td>0.940</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>0.102</td>
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<tr>
<td></td>
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<td><strong>0.006</strong></td>
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<td>0.960</td>
<td>2.6</td>
</tr>
<tr>
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<td>FH</td>
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<td>0.254</td>
<td>0.571</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
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<td>0.254</td>
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<td>-1.4</td>
</tr>
<tr>
<td></td>
<td>LS</td>
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<td>0.096</td>
<td>0.371</td>
<td>-1.2</td>
</tr>
<tr>
<td>Nr.Rods_V/Nr.Rods</td>
<td>FH</td>
<td>0.731</td>
<td>0.559</td>
<td>0.363</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>0.062</td>
<td>0.171</td>
<td>0.188</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td><strong>0.004</strong></td>
<td>0.054</td>
<td>0.393</td>
<td>4.2</td>
</tr>
<tr>
<td>⟨Ro_V.Th⟩</td>
<td>FH</td>
<td>0.444</td>
<td>0.285</td>
<td>0.133</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>0.737</td>
<td>0.420</td>
<td>0.598</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td><strong>0.031</strong></td>
<td>0.246</td>
<td>0.786</td>
<td>1.8</td>
</tr>
<tr>
<td>⟨Ro_H.Th⟩</td>
<td>FH</td>
<td>0.391</td>
<td>0.087</td>
<td>0.336</td>
<td>–</td>
</tr>
<tr>
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<td>0.078</td>
<td>0.490</td>
<td>0.496</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td><strong>0.005</strong></td>
<td>0.121</td>
<td>0.927</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*a* interaction term age:gender.

†*change per decade in percent.

‡bold numbers are smaller than 0.05.
The analysis of covariance revealed no differences between female and male bone samples (Table 3.2 and 3.3). Two exceptions were found for the ratio of the number of rods (Nr.Rods) to the number of plates (Nr.Plates) as well as $\langle Ro.\theta \rangle (p < 0.05)$. Both interactions appeared only in IC, the site with the highest variation. For the other sites we found no gender specific differences. Therefore, male and female data was pooled for further analysis and displayed in the same scatter plots (Figures 3.2 – 3.4).

From global morphometry, we found an age-related decrease in BV/TV which showed a linear relation in IC and LS. The decline was found to be 8.1%/decade (IC) and 6.4%/decade (LS). However, the variation was large at all ages. The sites differed in BV/TV being highest for FH and lowest for LS. A similar pattern was found for surface density (BS/TV) where a linear relation with age was found in LS only. This parameter seemed to decrease more dramatically at higher ages (> 70 years) similarly for all sites. Specific surface (BS/BV) on the other hand showed no age-related changes for any of the sites. This parameter was largest in LS and lowest in FH, which can be explained by the different size of the structure elements. There was a general increase in Tb.Sp which showed a linear relation with age in IC and LS. Again, the increase was more pronounced after the age of about seventy years. In LS and IC, spacing was found to be increased by 4.9%/decade and 6.2%/decade, respectively. Spacing was largest in LS and smallest in FH. Accordingly, Tb.N decreased with age and was largest in FH and smallest in LS. This parameter also showed a linear relation to age in IC and LS. No significant change with age was found for the globally determined trabecular thickness (Tb.Th) and the mean curvature of the trabecular bone structure ($\langle H \rangle$). The structure model index (SMI) increased slightly in LS only. These results are summarized in Figure 3.2 and Table 3.2.

From local morphometry, we found an age-related decrease in the number of rods in LS. On average a ninety-year-old person would have a 50% reduced number in rods as compared to a thirty-year-old subject. The other sites showed no obvious trend in this parameter. The ratio of the number of rods to the number of plates as well as the plate volume density (Pl.BV/BV) showed no age-related trend and the levels in Pl.BV/BV were quite different for the three sites. The slenderness of the rods ($\langle Ro.Sl \rangle$) increased with age and showed a linear relation in FH and LS. In IC no such linear relationship was found which is probably due to the large variation in bone microstructure in this site. The mean thickness of rods ($\langle Ro.Th \rangle$) and plates ($\langle Pl.Th \rangle$) showed a trend only in LS where an age-related increase was observed. The increase was observed in both, vertical ($\langle Ro_V . Th \rangle$) and horizontal ($\langle Ro_H . Th \rangle$)
elements. With increasing age, rods tended to be oriented along the anatomical axis (z-axis), which is reflected in LS by a linear increase of the percentage of vertical rods (Nr.Rods\textsubscript{V}/Nr.Rods). These results are summarized in Figures 3.3 and 3.4 and Table 3.3.

By performing the same statistical analysis including only the samples aged 59 years and older, all p-values became insignificant. The variation at these ages was obviously too large to reveal significant linear trends.

**Discussion**

This is the first study on age-related changes of three-dimensional (3D) local morphometric indices on human cancellous bone. Only recently a new method for the volumetric spatial decomposition of trabecular bone structures was proposed enabling local morphometry as applied on individual rods and plates [59-61]. Here we related these new indices to age, separately for men and women, in three different sites (femoral head, iliac crest, and lumbar spine). These sites were chosen since they represent plate-like, intermediate and rod-like bone structures. Additionally, to draw a picture of age-related bone changes, we also computed 3D global morphometric indices. Age-related changes of 3D global morphometric parameters have already been investigated for tibial cancellous bone [55,73]. However, we found no study, where global morphometry derived from micro-CT data was related to age in femoral head, iliac crest and lumbar spine. The aim of this study is to achieve a better understanding of age-related bone changes in the trabecular bone structure.

A separate analysis of bone samples from donors aged 59 years and older showed similar trends in all morphometric indices as the analysis of the whole dataset. However, due to the large variation at these ages, none of the linear regressions were significant anymore. This could indicate that age-related changes are very individual at a given age, but tend to be similar if compared over the whole lifetime. For this reason, in this study, only age-related changes over the whole lifetime were considered.

The first and most accepted age-related change in trabecular bone structure is bone loss as expressed in a decrease of calcified bone tissue. It is well accepted that such bone loss is an important factor leading to enhanced bone fragility and fracture risk in the elderly. Such bone loss has been demonstrated in many studies for different anatomical sites [11-13,18-23,26,27,39,55,74,75]. The results from our study are in line with these findings and we could demonstrate a linear relationship with age in IC and LS. According to our data, a ninety-years-old person showed a
3.1 Age-related changes in trabecular bone microstructures

Figure 3.2: Scatter plots with LOWESS fits for the global morphometric indices. The measured indices are bone volume density (BV/TV), bone surface (BS/TV), bone surface density (BS/BV), trabecular spacing (Tb.Sp), trabecular number (Tb.N), and trabecular thickness (Tb.Th). Open symbols denote female, and closed symbols denote male, where data from femoral head are displayed as circles (○, ●), data from iliac crest as triangles (△, ▲), and data from lumbar spine as squares (□, ■).
Figure 3.3: Scatter plots with LOWESS fits for the local morphometric indices. The measured indices are the number of rods (Nr.Rods), the ratio of the number of rods to the number of plates (Nr.Rods/Nr.Plates), the plate volume density (Pl.BV/BV), mean rod slenderness (\(\langle\text{Ro.Sl}\rangle\)), mean rod thickness (\(\langle\text{Ro.Th}\rangle\)), and mean plate thickness (\(\langle\text{Pl.Th}\rangle\)). Open symbols denote female, and closed symbols denote male, where data from femoral head are displayed as circles (○, •), data from iliac crest as triangles (△, ▲), and data from lumbar spine as squares (□, ■).
Figure 3.4: Scatter plots with LOWESS fits for the local morphometric indices. The measured indices are the mean orientation of rods ($\langle \text{Ro.}\theta \rangle$), the ratio of the number of vertical rods to the number of rods ($\text{Nr.Rods}_V/\text{Nr.Rods}$), the mean thickness of vertical rods ($\langle \text{Ro}_V.\text{Th} \rangle$) and the mean thickness of horizontal rods ($\langle \text{Ro}_H.\text{Th} \rangle$). Open symbols denote female, and closed symbols denote male, where data from femoral head are displayed as circles (○, ●), data from iliac crest as triangles (△, ▲), and data from lumbar spine as squares (□, ■).
decrease in BV/TV of 49% and 38% in IC and LS, respectively, as compared to a thirty-year-old subject. In FH we found no age-related changes although the lowess fit in Figure 3.2A tends to decrease at higher ages (> 70 years). It is a general finding of this study that age-related changes are clearer expressed in LS than in IC than in FH in all morphometric parameters. It is of course important to note that such age-related changes can only apply to an “average” population. Typical for cross-sectional studies, subject variation is too large to make statements about individual time courses.

Similar to BV/TV, bone surface density (BS/TV) in LS also decreased with age (6.8% per decade) in a linear fashion (Table 3.2). This is not surprising since larger volumes also have larger surfaces assuming a constant structure shape. The decrease was especially pronounced after the age of seventy (Figure 3.2B). Opposed to this, the specific bone surface (BS/BV), a measure for the relative amount of bone surface per bone volume, remained constant throughout life in all sites (Figure 3.2C, Table 3.2), a result which is supported by earlier findings from quantitative histology [25].

In clinics, biopsies from iliac crest are often used for diagnostics. However, this site is chosen for convenience reason and not because it is representative for the entire skeleton as already pointed out by other researchers [76]. From a morphometric point of view, we agree with this statement and strongly believe that 3D morphometric indices measured in this site must be considered very carefully for single biopsies. The reason for this is the intermediate hybrid structure type, as reflected by SMI [62] and Pl.BV/BV [61], leading to a high variation of morphometric parameters. This problem is even larger in cross-sectional studies in patients where it might be even difficult to harvest biopsies consistently from the exact same site in each person. However, in longitudinal studies using paired biopsies this problem can be avoided to some extend and these types of studies were able to reveal important person-specific changes in the iliac crest [77-80].

Trabecular spacing, number and thickness were investigated intensively with many different approaches. Concerning trabecular spacing there was not much controversy and to our knowledge all published results indicate an age-related increase [22,32,33,38-40,42,43], which is in line with our findings (Figure 3.2D, Table 3.2). Opposed to trabecular spacing, the trabecular thickness is of higher controversy and has thoroughly been investigated by means of two- and three-dimensional methods. In some studies it was found that the mean trabecular thickness decreased significantly with age [41-43], whereas in other studies no significant decrease could be observed [22,32]. Other studies found a significant decrease for mean horizontal trabecular thickness only, while the mean thickness of vertical trabeculae was un-
changed in vertebrae [38,40]. It was even speculated that vertical elements that are subjected to compression could increase in thickness as a compensatory response to loss of horizontal trabeculae [34,35]. However, as was pointed out earlier, the model-based derived thickness is underestimating the thickness compared to unbiased three-dimensional thickness measurements [62,73]. Nevertheless, direct three-dimensional thickness measurements showed a significant bilinear decrease with age [73].

In our study we approached the trabecular thickness on a 3D global as well as on a 3D true local level. Globally, we found no age-related changes in the trabecular thickness (Figure 3.2F, Table 3.2). On a local basis we investigated the thickness separately for plates and rods, which both showed an increase at higher ages in LS. This increase was found in both, vertical and horizontal elements (Figures 3.4B and 3.4C, Table 3.3). There are two interpretations to this result; first, trabeculae become thicker, second smaller trabeculae vanish. Since we also found in our study that the number of rods decreases with age we suggest that the second interpretation is more realistic and that at higher ages trabeculae first become thinner and finally vanish from the structure. This has important biological consequences since it was suggested that loss of trabeculae is more detrimental to bone strength than general thinning [58,81,82] and that eventually lost trabeculae can not be restored [36,37]. The latter is controversial since recent studies using parathyroid hormone (PTH) treatment showed an increase in connectivity density [83,84], whereas others found no such increase [85]. As was already pointed out, connectivity density is not a good measure since it cannot distinguish between changes in rod-like connections and fenestrated plates [58] and hence, from this measure it cannot be concluded whether new trabeculae are build by a certain treatment. For this, local methods would be better suited, since it becomes possible to actually count the number of trabeculae.

Increased trabecular spacing implies a decrease in trabecular number, which was found in earlier studies, where shortening or complete loss of trabeculae was documented [28,41]. In another study it was found that the trabecular number decreased significantly with age for both horizontal and vertical trabeculae [40]. In our study, we could demonstrate a loss of trabeculae with global and local morphometry. From global morphometry, we found a general loss of trabecular number in IC and LS. From local morphometry, we found a decrease in the number of rods (Nr.Rods) in LS only, whereas the other sites showed no age-related changes. For the LS this change is very obvious. The structures from this site are already in young people extremely rod-like and thus, if some elements are lost with age the number of rods
decreases as well. In FH and IC on the other hand the number of elements is more difficult to interpret. In aging, bones plates eventually get perforated which yields in new smaller plates and later in new rods. Thus, in FH and IC the number of elements increases due to plate perforation at the same time as some other elements vanish due to the aging process. Although bone remodeling mechanisms on a cellular level are most likely the same at both sites, they are expressed differently in the remodeling of the structure, due to the different initial structure types. This result is corroborated by a recent simulation study [86], where it was demonstrated that loss in BV/TV was linearly correlated to BS/BV, a measure for the structure shape.

It was suggested in different studies that buckling is an important failure mode [87-92]. The relative importance of this failure mode clearly increases with increasing slenderness of trabeculae. In our study, we found an increase in the mean slenderness of the rods $\langle \text{Ro.Sl} \rangle$, which was linear in LS and FH. This result is caused by a large increase in the length of the rods $\langle \text{Ro.Le} \rangle$, which was especially pronounced in LS.

In our study we could demonstrate that local morphometry used in combination with global morphometry may help to improve the understanding of age-related changes in trabecular bone microstructure. However, this study is limited due to the cross-sectional nature and the limited number of the samples resulting in a huge variation in morphometric parameters at any age. This was especially pronounced in IC, where it was hence difficult to formulate general age-related changes. In our opinion it might therefore be difficult to predict the overall state of the skeleton from this site for single biopsies [76]. However, IC was shown to yield important results in longitudinal studies with paired biopsies [77,79,80,84] and we would expect local morphometry to be a helpful tool for follow-up studies also in IC in order to find out which structural elements were affected the most by age, disease or treatment. Additionally, the presented method requires imaging systems that are able to acquire trabecular microstructure at a relatively high resolution. Also for this reason, IC biopsies may be important in future follow-up studies since they can actually be measured ex vivo by means of very high resolution µCT. Assessment of bone microstructure in vivo is a challenging task. However, studies on the human forearm showed the possibility to assess trabecular microstructure at the level of about 100 µm and proved to yield reasonable results in follow-up studies [93].

We would like to summarize our findings as follows. 1) Age related changes are best seen in LS. The reason for this is the extremely rod-like structure type which is expressed by a high specific bone surface that is sensitive to both, hormonal and
environmental changes. In aging people, thin rods are further thinned and finally vanish from the structure which causes a transformation of the trabecular bone structure to longer and on average thicker rods. Due to loss of interconnecting rods, the remaining trabeculae are more likely to buckle increasing the potential for catastrophic failure. 2) Due to the high variation in almost all morphometric indices in IC at all ages it seems difficult to predict only from this site the state of the overall skeleton in cross-sectional studies. However, follow-up studies with paired biopsies might still be important because they have been shown to reveal important group differences. 3) Trabecular bone from FH is extremely plate-like at all ages. Changes in these structures are expressed by a simultaneous thinning and loss of interconnecting trabeculae and perforation of plates leading to new plates and rods. With these changes connectivity density can either increase or decrease and is therefore not a representative measure for the state of the bone.

In conclusion, our work demonstrates that 3D local morphometry in combination with 3D global morphometry are useful tools for the investigation of changes in trabecular bone microstructures. In our study these tools were used to improve our understanding of age-related microstructural changes in trabecular bone, but these new tools could in future studies also help to better understand microstructural changes related to disease and treatment in individual rod and plate elements separately.

Acknowledgements

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References


3.1 Age-related changes in trabecular bone microstructures


3.1 Age-related changes in trabecular bone microstructures


3.1 Age-related changes in trabecular bone microstructures


3.2 Importance of individual rods and plates in the assessment of bone quality and their contribution to the competence of bone

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submitted to:
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Abstract:
Local morphometry based on the assessment of individual rods and plates was applied to forty-two human vertebral trabecular bone samples. Results showed that models based on local morphometry as a measure for bone quality helped improving our understanding of the role of local structural changes in the determination of bone competence as assessed from direct and computational biomechanics.

In a recent study, we proposed a method for local morphometry of trabecular bone, i.e. morphometry as applied to individual rods and plates. In this study, we used this method to investigate the relative importance of local morphometry in the assessment of bone quality and its relative contribution to the competence of human vertebral bone.

We extracted forty-two human trabecular bone autopsies from 9 intact spinal columns. The cylindrical samples were imaged with micro-computed tomography (\(\mu\)CT) to assess bone microstructure. From these images, global and local morphometric indices were derived and related to Young’s modulus as assessed by experimental uniaxial compression testing (\(E_{\text{meas}}\)) and computational finite element analysis (\(E_{FE}\)).
We found the best single predictor for Young’s modulus to be apparent bone volume density (BV/TV), which explained 89% of the variance in $E_{FE}$ when fitted with a power law. A multiple linear regression model combining mean trabecular spacing (Tb.Sp), mean slenderness of the rods ($\langle \text{Ro.Sl} \rangle$), and the relative amount of rod volume to total bone volume (Ro.BV/BV) was able to explain 90% of the variance in $E_{FE}$. This model could not be improved by adding BV/TV as an independent variable. Furthermore, we found that mean trabecular thickness of the rods was significantly related to $E_{FE}$ ($R^2 = 0.42$), whereas mean trabecular thickness of plates had no correlation to Young’s modulus. Since the globally determined trabecular thickness does not discriminate between rods and plates, this index had only a poor predictive power for $E_{FE}$ ($R^2 = 0.09$) demonstrating the importance of local analysis of individual rods and plates.

From these results, we conclude that models based on local morphometry help improving our understanding of the relative importance of local structural changes in the determination of the competence of bone. Separate analysis of individual rods and plates may help to better predict age and disease related fractures as well as to shed new light on the effect of pharmaceutical intervention in the prevention of such fractures beyond bone density.

**Keywords:**
local morphometry, Young’s modulus, finite element method, bone quality, trabecular bone, trabecular plates, trabecular rods, vertebrae

**Introduction**

Osteoporosis is now recognized as one of the major public health problems facing postmenopausal women and aging individuals irrespective of gender [1]. It is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. Bone strength reflects the integration of two main features: bone mineral density expressed as grams of mineral per area or volume and bone quality referring to bone architecture, turnover, damage accumulation, collagen cross-linking and mineralization [2]. To assess bone mechanical properties, different experimental testing methods have been proposed [3]. For trabecular bone, compression [4-9], and tensile testing [5,7] were used to assess apparent Young’s modulus and ultimate strength. Additional to these experimental methods, the stiffness and strength of trabecular bone samples was also computed by finite element
model simulations [10-12], which showed to yield qualitatively similar results as experimental approaches.

The assessment of bone strength has traditionally been related to surrogate measures potentially predicting and explaining the variation in stiffness and strength. Among those, the most established one certainly is bone volume density (BV/TV), which has been shown to explain a great amount of the variance in both stiffness and strength when fitting a power law [13-15]. However, it has also been recognized that although older persons may lose bone as expressed by a decrease in bone density, they do not develop fractures. This is not necessarily unexpected since bone mineral density, geometry of bone, micro-architecture of bone and quality of the bone material are all components that determine bone strength [16,17]. For this reason, efforts in the quantification of structural properties gained in importance and many different methods have been proposed [18]. Among the basic parameters were the measurement of bone volume (BV) and bone surface (BS), which can be derived from volumetric and surface meshes, respectively [19,20]. Bone volume density (BV/TV) or specific bone surface (BS/BV) can then be derived from these primary measures. Additionally, the mean trabecular thickness (Tb.Th), the mean trabecular separation (Tb.Sp), and the trabecular number (Tb.N) are often determined parameters that can be computed directly from the three-dimensional image without any model assumption [21]. Furthermore, the connectivity density (Conn.D) was introduced to characterize the three-dimensional trabecular network [22]. To estimate the plate-rod characteristic of a trabecular bone structure a parameter called structure model index (SMI) was invented [23], which was shown to be closely related to the mean curvature \( \langle H \rangle \) of the bone surface [24]. Also related is the trabecular bone pattern factor (TBPf) [25], which equals \( 2 \langle H \rangle \). Mean intercept length (MIL) and other measures of architectural anisotropy (DA) such as volume orientation, star volume distribution or star length distribution [18] were used to improve the prediction of multiaxial elastic properties of trabecular bone from bone volume density alone [26].

A common feature to all these studies is that they were applied to the trabecular structures as a whole and hence were not able to capture structural changes on an elemental level, i.e. a single rod or plate. Only few attempts have been made to investigate local parameters of the trabecular network. Pothuaud et al [27] presented a method called line skeleton graph analysis (LSGA) to compute topological parameters as well as the length and volume of single trabecular elements. They showed that LSGA can be applied \textit{in vivo} [28] and had the potential to improve the prediction of mechanical properties when combined with bone volume fraction [29]. Their method however, was based on a line-skeleton where shape information was
lost and an identification of plates and rods was not possible. An attempt to also assess shape information was done by Saha et al [30], who first introduced a method for the digital topological characterization of the trabecular bone architecture. Their method is based on an thinning algorithm [31] followed by a classification algorithm [32] and allowed them to subdivide the trabecular structure into its rods and plates. This method was later used for orientation analyses of the trabecular bone networks [33] and it could be shown that the locally determined orientations better described anisotropy than the mean intercept length (MIL). Only recently, we presented a new approach, conceptually combining the three-dimensional identification of trabecular elements [27] with the classification of shape preserved skeletons [32]. With this method the structural elements (i.e. rods and plates) could be analyzed in their full three-dimensional and volumetric extent, which was referred to as local bone morphometry [34].

The goal of this study was to investigate the relative importance of local morphometry in the assessment of bone quality and its relative contribution to the competence of human vertebral bone. For this, we related local and global morphometric indices to the apparent Young’s modulus as assessed by combined experimental uniaxial compression testing and computational finite element analysis.

Methods

Direct mechanical testing

Forty-two human trabecular bone samples were extracted from intact spinal columns of nine donors who took part in an anatomical donation program. A specially developed drill was used to core the cylindrical specimens (10 mm height and 8 mm in diameter) from lumbar and thoracic vertebrae under constant water irrigation. Preceding drilling, contact radiographs were taken from each vertebra to orient them into a position such that the main trabecular orientation was aligned with the longitudinal axis of the cored specimen. No data was available on age and disease state of the donors. The specimens were cleaned from soft tissue, embedded in PMMA to minimize end-artifacts, and after 5 preconditioning cycles, tested in compression with a speed of 0.05%/s. An external extensometer was used to measure strain over a length of 9.3 mm. The apparent Young’s modulus ($E_{meas}$) was then calculated form the stress-strain curve.
Assessment of bone microarchitecture

Before mechanical testing, all specimens were scanned using a micro-tomographic system ($\mu$CT40, Scanco Medical AG, Bassersdorf, Switzerland) with a nominal resolution of 20 $\mu$m, to assess the trabecular bone architecture. The reconstructed images were filtered using a constrained 3D Gaussian filter to partially suppress noise in the volumes ($\sigma = 1.2$ voxel, support = 1 voxel), and binarized using a global threshold (22.4%). A cylindrical region with a diameter of 7 mm was digitally cut, to exclude bone fragments that might have resulted from the cutting process. Component labeling was performed to remove unconnected parts of the structure.

Finite element analysis

From the component labeled images, finite element (FE) models consisting of identical hexahedral elements were created using a standard voxel conversion technique. To decrease computational time, but still ensuring accurate outcomes, the voxel size was reduced to 40 $\mu$m, which is about one fourth of the mean trabecular thickness, as recommended for numerical convergence [35]. To represent the experimental set-up, the nodes on the bottom plane were fully fixed, whereas the nodes on the top surface underwent an axial displacement to obtain 1% apparent strain. The FE models were then used to calculate the force needed to achieve this displacement. From this data, the apparent Young’s modulus ($E_{FE}$) was computed and scaled ($E_{tissue} = 10.7$ GPa) such that the slope of $E_{meas}$ vs. $E_{FE}$ equaled one. To solve the models an element-by-element method [36] was used running on a HP Superdome System with 64 RISC (550 MHz) processors.

Global morphometry

We used standard three-dimensional morphometry to compute relative bone volume density (BV/TV), bone surface density (BS/TV), and specific bone surface (BS/BV). These indices were derived from a triangulated mesh allowing for computation of surface and volume [19,20]. Furthermore, the mean curvature ($\langle H \rangle$) [24] which is proportional to the trabecular bone pattern factor (TBPf = $2\langle H \rangle$), and the structural model index (SMI) [23] as well as the mean trabecular thickness (Tb.Th), the mean trabecular separation (Tb.Sp) [21], and the degree of anisotropy (DA) were computed. All theses indices were computed directly from the component labeled images without any inherent model assumption.
Local morphometry

The component labeled images were then skeletonized to a homotopic and shape preserving one-voxel-thick skeleton, followed by a topological optimization procedure [34]. The optimized skeleton was then characterized by a slightly modified point-classification algorithm originally devised by Saha et al [32]. Based on this classification, the skeleton was spatially decomposed into rods and plates, where rods were identified to be the elements having exactly two nodes. The elements were then expanded to their original size by applying a so called multi-color dilation algorithm [34]. This operation resulted in a spatially decomposed bone structure where all rods and plates were labeled with an individual color (Figure 3.5).

From this decomposed structure we computed local morphometric indices, which means that we applied standard three-dimensional morphometry to all rods and plates individually on a true elemental level. In order to follow the naming conventions proposed by Parfitt et al [39] we previously suggested to use the prefix Pl for plates, and Ro for rods. The volume, surface, and thickness of one single element were denoted with V, S, and Th, and the sum of the volume and surface over all elements with BV, and BS, respectively. Furthermore, we use brackets (⟨⟩) to denote mean values that are averaged over all elements of the same type.

In this study we concentrated on the mean volume (⟨Ro.V⟩, ⟨Pl.V⟩), the mean surface (⟨Ro.S⟩, ⟨Pl.S⟩), as well as the mean thickness (⟨Ro.Th⟩, ⟨Pl.Th⟩) averaged for each structure over all rods and plates separately. Additionally, the mean curvature (⟨Ro.H⟩, ⟨Pl.H⟩) as computed only on the exposed surface (without interface surface) was computed. For the rods we also computed the mean slenderness (⟨Ro.Sl⟩) and mean orientation (⟨Ro.θ⟩). Furthermore, the percent plate (Pl.BV/BV) and rod volume fraction (Ro.BV/BV = 100% – Pl.BV/BV) were determined.

Statistical analysis

The experimentally measured Young’s modulus (E_{meas}) was linearly related to the FE derived Young’s modulus (E_{FE}) and the Pearson correlation coefficient was computed to test for the relative deviation of the two methods (Figure 3.6). Further, BV/TV was related to both moduli by fitting a power law (Figure 3.7). Young’s modulus as predicted by BV/TV ($E_{pred}(BV/TV)$) was also plotted versus $E_{meas}$ and compared to the log transformed data of $E_{meas}$ and BV/TV (Figure 3.8). All global and local morphometric indices were related to $E_{FE}$ using linear and power laws as appropriate (Table 3.4 and 3.5). Multiple linear regression analysis was done with
Figure 3.5: Spatial decomposition of trabecular bone. A and B show a rod- and plate-structure, respectively, where each trabecula is coded by its color. C and D show the same structures but display rods in blue and plates in red.
Table 3.4: Correlation of global morphometric parameters versus the finite element Young’s modulus ($E_{FE}$).

<table>
<thead>
<tr>
<th>Index</th>
<th>Model</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV/TV</td>
<td>power</td>
<td>0.89*</td>
</tr>
<tr>
<td>BS/TV</td>
<td>linear</td>
<td>0.83*</td>
</tr>
<tr>
<td>BS/BV</td>
<td>power</td>
<td>0.75*</td>
</tr>
<tr>
<td>SMI</td>
<td>linear</td>
<td>0.74*</td>
</tr>
<tr>
<td>$\langle H \rangle$</td>
<td>power</td>
<td>0.69*</td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>linear</td>
<td>0.62*</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>linear</td>
<td>0.09‡</td>
</tr>
<tr>
<td>DA</td>
<td>linear</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* $p < 0.001$
‡ $p < 0.05$
n.s. not significant

global and local morphometric indices to find a model that accurately predicted $E_{FE}$. For all statistical analyses, the GNU statistical computation and graphics package R (Version 2.0.1; http://www.r-project.org) was used.

Results

The Young’s modulus as assessed by experimental two platen compression testing ($E_{meas}$) was in good agreement ($R^2 = 0.85$) with the Young’s modulus as assessed by

![Figure 3.6: The Young’s modulus from the finite element analysis ($E_{FE}$) was in good agreement ($R^2 = 0.85$) with the Young’s modulus from the measurement ($E_{meas}$).](image-url)
Figure 3.7: BV/TV was in good agreement with measured and FE-determined apparent modulus. A: $E_{\text{meas}}$ ($R^2 = 0.87$) and B: $E_{FE}$ ($R^2 = 0.89$). Dashed lines show the confidence interval and dotted lines show the prediction interval.

standard finite element simulation ($E_{FE}$). However, the fit was not perfect (Figure 3.6) due to the relatively large voxel-size (40 µm) as compared to the trabecular thickness, which introduces errors that are especially pronounced in low bone volume fraction samples, and due to measurement errors in the experimental approach.

Bone volume density (BV/TV) predicted $E_{\text{meas}}$ ($\sim$BV/TV$^{1.81}$; $R^2 = 0.87$) and $E_{FE}$ ($\sim$BV/TV$^{1.65}$; $R^2 = 0.89$) equally well by fitting a power law. The prediction

Figure 3.8: A: In the log-transformed data the relation $E_{\text{meas}} \sim \text{BV/TV}$ becomes linear and the variance is constant over the range. B: If the relation found from the log-transformed data is applied ($E_{\text{pred}}(\text{BV/TV})$) the variation is not constant over the range anymore wherefore no simple linear relation can be fitted.
Table 3.5: Correlation of local morphometric parameters versus the finite element Young’s modulus ($E_{FE}$).

<table>
<thead>
<tr>
<th>Index</th>
<th>Model</th>
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<tr>
<td>$\text{Ro.BV/TV}$</td>
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<td>0.58*</td>
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<tr>
<td>$\text{Pl.BV/TV}$</td>
<td>linear</td>
<td>0.44*</td>
</tr>
<tr>
<td>$\text{Ro.BV/BV}$</td>
<td>linear</td>
<td>0.31*</td>
</tr>
<tr>
<td>$\text{Pl.BV/BV}$</td>
<td>linear</td>
<td>0.31*</td>
</tr>
<tr>
<td>$\langle \text{Ro.BV} \rangle$</td>
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<td>0.40*</td>
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<tr>
<td>$\langle \text{Ro.BS} \rangle$</td>
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<td>n.s.</td>
</tr>
<tr>
<td>$\langle \text{Ro.Th} \rangle$</td>
<td>linear</td>
<td>0.42*</td>
</tr>
<tr>
<td>$\langle \text{Ro.} \theta \rangle$</td>
<td>linear</td>
<td>0.39*</td>
</tr>
<tr>
<td>$\langle \text{Ro.Sl} \rangle$</td>
<td>linear</td>
<td>0.51*</td>
</tr>
<tr>
<td>$\langle \text{Ro.} \langle \text{H} \rangle \rangle$</td>
<td>linear</td>
<td>0.80*</td>
</tr>
<tr>
<td>$\langle \text{Pl.BV} \rangle$</td>
<td>linear</td>
<td>0.20†</td>
</tr>
<tr>
<td>$\langle \text{Pl.BS} \rangle$</td>
<td>linear</td>
<td>0.17†</td>
</tr>
<tr>
<td>$\langle \text{Pl.Th} \rangle$</td>
<td>linear</td>
<td>n.s.</td>
</tr>
<tr>
<td>$\langle \text{Pl.} \langle \text{H} \rangle \rangle$</td>
<td>linear</td>
<td>0.09‡</td>
</tr>
</tbody>
</table>

* $p < 0.001$
† $p < 0.01$
‡ $p < 0.05$

not significant

Intervals in Figure 3.7 nicely demonstrate that the variance increases with increasing $\text{BV/TV}$. In the log-transformed data, where the Pearson correlation coefficient has its validity, the data had a constant variance over the whole range (Figure 3.8A). To demonstrate the effect of increasing variance, the power law was used to compute the predicted modulus ($E_{\text{pred}}(\text{BV/TV})$), which was plotted versus $E_{\text{meas}}$ (Figure 3.8B). In this linear model only 70% percent of the variance in $E_{\text{meas}}$ could be explained by the $\text{BV/TV}$-based stiffness prediction.

For the remainder of the analyses only relationships with $E_{FE}$ are presented, since our aim was to assess the effects of microstructure on bone competence. Since the morphometric indices and $E_{FE}$ were derived from the same digital images we could also prevent introducing unwanted variance due to measurement errors as they are implicitly included in $E_{meas}$ and due to errors in image processing like thresholding of the images.

The correlations of global and local morphometric indices versus $E_{FE}$ are shown in Table 3.4 and Table 3.5, respectively. Apart from Tb.Th and DA all global indices showed a good correlation with $E_{FE}$ and explained more than 60% of the variance. Best single predictors were $\text{BV/TV}$ by fitting a power law and $\text{BS/TV}$ by using
a linear fit. These two parameters were also in excellent agreement ($R^2 = 0.94$) with each other and hence not independent. Other important interdependencies were found for the following pairs; SMI vs. $\langle H \rangle$ ($R^2 = 0.93$), BS/TV vs. Tb.Sp ($R^2 = 0.85$), BV/TV vs. Tb.Sp ($R^2 = 0.77$), and BV/TV vs. $\langle H \rangle$ ($R^2 = 0.77$).

From local morphometry, it was found that the mean curvature of the rods was the best single predictor with an excellent correlation to $E_{FE}$ ($R^2 = 0.80$). The predictive power of the other local morphometric indices was generally lower, where indices derived from rods explained in average 40% to 50% but indices derived from plates up to only 20% of the variance in $E_{FE}$. Moreover, multiple linear regression analysis revealed that a model based on trabecular spacing, slenderness of the rods, and the percent rod volume fraction was able to explain 90% of the variance in $E_{FE}$ in a very linear fashion (Figure 3.9). This correlation could not be improved by adding BV/TV as an additional independent variable.

**Discussion**

In this study we related the elastic properties of human trabecular bone samples to local and global morphometric indices. Only recently, a new method has been proposed for the volumetric spatial decomposition of trabecular bone structures enabling local morphometry as applied on individual rods and plates [34]. In this study we relate these new indices to bone stiffness to investigate the relative importance of structural differences between trabecular elements. These differences could be

![Figure 3.9: $E_{pred} = 1745 - 121Ro.Sl - 637Ro.BV/BV - 394Tb.Sp$. The model was in very good agreement ($R^2 = 0.90$) with the Young’s modulus as derived from the finite element analysis ($E_{FE}$).](image)
analyzed separately for rods and plates.

Bone stiffness was assessed by standard compression testing as well as by finite element model simulation. The Young’s modulus of these two independent methods was in good agreement \((R^2 = 0.85)\). However, the fit was not perfect and Figure 3.6 implies that the relation could also have been modeled as a power law. This can be explained by the two limitations of the models. First, the mechanical compressions testing technique is not perfect and the concept of effective elastic modulus depends on many factors such as specimen size and geometry \([4,6]\), boundary conditions \([9]\), and strain rate \([40]\). Thus, in mechanical compression experiments there is always an intrinsic error that cannot be neglected and may result in a relatively high variation (mostly damage of the sample during preparation, bending of the sample during compression, precise location and gauge length of the strain measure). Second, our finite element model simulation only accounted for structure and did not include variations in local material properties of the bone matrix. Additionally, the geometry of the FE model is highly sensitive to image segmentation and thin structures may behave too stiff in bending. For these two reasons it is clear that both moduli deviate from the true apparent Young’s modulus, and that \(E_{meas}\) and \(E_{FE}\) do not necessarily build a linear relationship. Since the global and local morphometric parameters investigated in our study are derived from digital images we chose to compare them to \(E_{FE}\); hence, our findings are not biased by material properties but only take into account structural effects.

Young’s modulus of human vertebral trabecular bone samples could accurately be predicted by combining mean trabecular spacing (Tb.Sp), mean slenderness of the rods (⟨Ro.Sl⟩), and the relative rod volume fraction (Ro.BV/BV) in a multiple linear regression model. The presented model was able to explain 90% of the variance in \(E_{FE}\), where all three parameters were negatively correlated with the FE-based stiffness. This means that increased trabecular spacing yields in a decrease in bone stiffness; an intuitive result. An increase in the slenderness of the rods also decreased trabecular bone strength. Also this result is not unexpected, since slender rods are more exposed to bending or buckling than thick, short rods. An increase in these first two parameters are likely caused by loss of trabeculae. The third parameter showed that an increase in the relative amount of rod volume in percentage of bone volume had a negative effect to bone stiffness. It has been proposed that in aging, the reduction in bone volume is mainly due to a reduction in plate density, where the process of plate-removal was initiated by an excessive depth of osteoclastic resorption cavities, leading to focal perforation of plates, followed by progressive enlargement of the perforation with conversion of plates to rods \([41,42]\).
In such a process Ro.BV/BV would clearly increase and hence lead to a decrease in bone strength. It is noteworthy that the proposed model only included structural information not directly related to bone density or bone mass; even more so, BV/TV or BV did not add predictive power to this model.

Since bone density plays an important role in the prediction of the mechanical behavior of trabecular bone and can be assessed relatively easily at different sites of interest, it is still subject of many investigations. Nevertheless, the precise relation between Young’s modulus and BV/TV remains still controversial [43] and has been modeled by power laws with exponents ranging from 1 to 3 [13-15,44,45]. In our study, we used a power law to predict both $E_{\text{meas}}$ and $E_{\text{FE}}$, resulting in equally good correlations (Figure 3.7). The exponents were found to be 1.81 for $E_{\text{meas}}$ and 1.65 for $E_{\text{FE}}$, respectively. However, the correlation plots showed heteroscedasticity (increasing variance with increasing BV/TV) which is nicely illustrated in Figure 3.7 where the variance increased with increasing BV/TV. This is also obvious in Figure 3.8B, where the determined power law was applied to the data to predict Young’s modulus ($E_{\text{pred}}(BV/TV)$). After transformation, the linear model only explained 70% of the variance in $E_{\text{meas}}$, as compared to 87% when fitting the log-transformed data (Figure 3.8A). For this reason BV/TV will typically lack predictive power when used to assess bone stiffness in real applications of strength prediction.

From global morphometry we found that besides BV/TV also bone surface density (BS/TV), specific bone surface (BS/BV), structure model index (SMI), mean curvature ($\langle H \rangle$), and trabecular separation (Tb.Sp) all had a high predictive power and were able to explain more than 60% of the variance in $E_{\text{FE}}$ (Table 3.4). However, none of these parameters was as good as BV/TV alone and we could not find a multiple linear regression model that could improve the prediction power much over the predictive power over that of BV/TV alone. A reason for this may lie in the fact that many of the global morphometric indices were in excellent agreement with each other ($R^2 > 0.77$), hence, indicating a lack of parameter independence on the global level.

From local morphometry we found the best single predictor for $E_{\text{FE}}$ to be the mean curvature of the rods ($\langle \langle \text{Ro.} \langle H \rangle \rangle \rangle$). This relation ($R^2 = 0.80$) was negative, which means that as rods are transformed from a relatively flat element to a perfect cylindrical element with circular cross-section, the trabecular bone structure becomes less stiff. Such a transformation will appear as a result of thinning of the rods [41,42]. The other local morphometric indices were relatively moderate single predictors for $E_{\text{FE}}$, where in general indices derived from rods showed better correlations to $E_{\text{FE}}$ than indices derived from plates (Table 3.5). It is noteworthy that
the mean trabecular thickness as measured by global morphometry was only poorly correlated to $E_{FE}$, whereas the mean trabecular thickness of the rods was able to explain 42% of the variance in $E_{FE}$. Furthermore, the mean trabecular thickness of the plates showed no significant correlation at all. Since the computation of global Tb.Th does not discriminate between rods and plates this measure is averaged over the whole structure and all element types. This is a strong indication that it is important to also include local morphometry in the characterization of trabecular bone samples, to look at individual contributions of rods and plates to the competence of bone. Although the degree of anisotropy (DA) had no predictive power for bone competence in this study (samples were perfectly aligned to the principle axis of loading), it might be important to include DA and/or other global parameters in a combined index for the prediction of stiffness along other material directions.

In conclusion, here we demonstrated for the first time that although bone density or mass is the best single predictor of bone stiffness, it is not required to predict bone stiffness when structural properties are incorporated in the analysis. A multiple linear regression model based on locally and globally determined structural information was able to predict the axial Young’s modulus of human vertebral trabecular bone samples independent of bone density or bone mass. Although indices based on density or mass and indices based on local structural measures performed almost the same in the prediction of stiffness, these newly proposed local parameters have clear advantages over traditional density-based measures. They allow assessment of why and how a structure is changing, for example in the course of a certain pathology or with treatment. One can imagine scenarios where bone density is changing only mildly after treatment whereas changes of bone competence can be very pronounced. Local morphometry might be a tool to explain those disproportional changes in bone change as a new measure of bone quality and the competence of bone. In that sense, separate analysis of individual rods and plates may help to better predict age and disease related fractures as well as to shed new light on the effect of pharmaceutical intervention in the prevention of such fractures beyond bone density.

Acknowledgements

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References


3.2 Contribution of rods and plates to bone quality and competence


3.3 Limitations of morphometric indices in the prediction of trabecular bone failure behavior in uniaxial compression testing

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Abstract:
Since it is well accepted that bone volume density (BV/TV) can not explain the whole variance in bone strength, many efforts in finding independent measures for an accurate and reliable prediction of bone mechanical properties have been done, which lead to the development of many morphometric indices characterizing trabecular bone microstructure. Generally, these indices assume a high homogeneity within the bone sample. However, in the present study we could show that the variance in BV/TV in a single bone sample can be relatively large (CV = 9.07\% – 28.23\%). To assess the limitations of morphometric indices in samples where the assumption of homogeneity is not met, we extracted 13 autopsies of a single human spine. The cylindrical samples were measured by image guided failure assessment (IGFA), a technique combining step-wise micro-compression and micro-computed tomography (\(\mu\)CT). Additionally, we computed morphometric indices for the whole sample as well as for ten equal subregions along the anatomical axis. We found that ultimate strength was equally well predicted by BV/TV of the whole sample \(R^2 = 0.55\) and BV/TV of the weakest subregion \(R^2 = 0.57\). Investigating three-dimensional animations of structural bone failure, we could demonstrate that two main failure mechanisms determine the competence of trabecular bone samples;
in homogeneous trabecular bone samples, competence is determined by a whole set of trabecular elements, whereas in inhomogeneous bone samples a single or a missing trabeculae may induce catastrophic failure. The later failure mechanism cannot be captured by conventional morphometry.

**Keywords:**
Morphometry, compression testing, limitations, autopsies, biopsies

**Introduction**

Osteoporosis is now recognized as one of the major public health problems facing postmenopausal women and aging individuals irrespective of gender [1]. It is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. Bone strength reflects the integration of two main features: bone mineral density expressed as grams of mineral per area or volume and bone quality referring to bone architecture, turnover, damage accumulation, collagen cross-linking and bone mineralization [2].

To assess bone strength, different experimental testing methods have been proposed [3]. For trabecular bone biopsies and autopsies, compression [4-9], and tensile testing [5,7] were used to assess apparent Young’s modulus and ultimate strength. Compression testing was recently expanded to a method called image guided failure assessment (IGFA) [10]; a method which incorporates step-wise micro-compression in combination with time-lapsed micro-computed tomography (µCT). This method allows for the assessment of mechanical data in addition to providing three-dimensional images that enable to visually study the three-dimensional failure behavior of cellular solids [11,12].

The assessment of bone strength has traditionally been related to independent measures potentially predicting and explaining the variation in stiffness and strength. Among those, the most established one certainly is bone volume density (BV/TV), which has been shown to explain a great amount of the variation in both stiffness and strength when fitting a power law [13-15]. However, it has also been recognized that older persons may lose bone as expressed by a decrease in bone density, but do not develop fractures, since bone mineral density, geometry of bone, micro-architecture of bone and quality of the bone material are all components that determine bone strength [16,17]. For this reason, efforts in the quantification of structural properties gained in importance and many different methods have been
proposed to further describe the influence of changes in bone microstructure on its mechanical properties [18-26]. However, these methods typically present averaged numbers for the entire specimen where inhomogeneities and local variations in these indices cannot be captured. It may be these inhomogeneities which may locally weaken the trabecular bone structure and finally initiate failure. This raises the question on how reliable failure prediction based on morphometric indices can be and how results from compression testing experiments must be interpreted.

In this study we used results from IGFA experiments in combination with morphometric indices to analyze the relative effect of local bone structure variations on the mechanical properties of trabecular bone samples. We aimed to visually identify differences in failure modes for different structure types. We think that these observations show some limitations in the strength prediction of whole bones, which is based on uniaxial compression testing experiments or morphometry of autopsy and biopsy samples.

Methods

A group of 13 human vertebral cancellous bone specimens were cored (Ø 7.85 ± 0.21 mm) from thoracic and lumbar regions of vertebral bodies harvested from the spine of one donor (65 y/o M) participating in the Harvard Anatomical Gift program. The bone specimens were cored parallel to the anatomical axis out of a pre-cut block of the vertebral body using a diamond coring-tool (Starlite Industries, Rosemont, PA) while completely submerged in 0.9% saline solution. All specimens were stored in saline-soaked gauze at a temperature of −20°C. Once cored, the two ends of all specimens were cut perpendicular to the cylindrical axis between two parallel diamond blades running on a low-speed saw (Isomet, Buehler Corp., Lake Bluff, IL) operating under saline irrigation (H 11.62 ± 0.14 mm).

Before testing, pre-aligned brass end caps (Ø 9 mm, H 1.2 mm) were glued to both ends of the specimens. This step effectively reduced end artifacts by restraining displacement at either end of the specimen and by providing support to the free ends of the free elements [27]. The specimens remained wet during testing with the humidity sealed within the micro-compression device, which was verified upon retrieval of wet specimens at the end of testing periods.

To assess the data, we used a previously described novel mechanical testing and data acquisition (MTDAQ) device [11,12]. This method incorporates step-wise micro-compression in combination with time-lapsed micro-computed tomographic
imaging ($\mu$CT) to study the 3D failure behavior of cellular solids, a method previously referred to as image guided failure assessment (IGFA) [10]. All specimens were preconditioned to eliminate typical toe behavior [6,28] at a strain rate of 0.005 s$^{-1}$ for 7 cycles. The specimens underwent sequential compressive steps of 0%, 4%, 8%, 12%, 16% and 20% nominal strain.

Progressive images were generated using a micro-tomographic imaging system ($\mu$CT 20, Scanco Medical AG, Switzerland), a compact fan-beam type tomograph, also referred to as desktop $\mu$CT [29]. This specific system with an isotropic spatial resolution of 28 $\mu$m has been used extensively for different research projects involving the assessment and analysis of microstructural bone and porous materials [29-36].

Measurements were stored in three-dimensional image arrays with isotropic voxel sizes of 34 $\mu$m. A three-dimensional Gaussian filter with a limited, finite filter width and support was used to partially suppress noise in the volumes. These images were binarized to separate bone from background using a global thresholding procedure [37]. A component labeling algorithm was applied to keep only the largest connected bone-component and to remove small particles arising from noise and artifacts.

Each specimen’s $\mu$CT image was divided along the cylinder axis into 10 sub-regions of equal height (Figure 3.10). For each of these sub-regions as well as for the whole specimen conventional morphometric indices were computed. The morphometric indices obtained were as follows: bone volume density (BV/TV), specific bone surface density (BS/BV) [18], trabecular bone pattern factor (TBPf) [24], trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp) [20], degree of anisotropy (DA), and connectivity density (Conn.D) [21].

The 3D images of each compression step were combined into an animation, since 3D animations of the mechanical experiments contribute significantly to the understanding of specimen failure. For this purpose the 3D images had to be aligned initially with respect to the bottom end-plate, since this plate was fixed during the experiment. An algorithm was used to find the last plane of this end-plate in each 3D image enabling an alignment of the images along the perpendicular axis. A subsequent 2D correlation procedure was used in the first five bottom planes in order to perform alignment. Each aligned 3D dataset was then visualized under the same conditions (orientation, light settings) by using an extended Marching Cubes algorithm [38]. The resulting images were finally turned into an animation to visualize failure for all specimens, where a selection is shown in Figures 3.11 and 3.12. This procedure was repeated for four main directions (front, left, back, right) for the whole specimen as well as for two directions (front, left) for 110 central coronal slices (3.74 mm).
Table 3.6: Global morphometry

<table>
<thead>
<tr>
<th></th>
<th>BV/TV</th>
<th>BS/BV</th>
<th>SMI</th>
<th>TBpf</th>
<th>DA</th>
<th>Tb.Th</th>
<th>Tb.Sp</th>
<th>Conn.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>4.19</td>
<td>15.44</td>
<td>1.62</td>
<td>4.30</td>
<td>1.21</td>
<td>0.146</td>
<td>1.011</td>
<td>0.58</td>
</tr>
<tr>
<td>max</td>
<td>12.33</td>
<td>20.79</td>
<td>2.18</td>
<td>7.12</td>
<td>1.53</td>
<td>0.204</td>
<td>1.589</td>
<td>2.15</td>
</tr>
<tr>
<td>mean</td>
<td>7.82</td>
<td>17.95</td>
<td>1.92</td>
<td>5.77</td>
<td>1.40</td>
<td>0.170</td>
<td>1.266</td>
<td>1.37</td>
</tr>
<tr>
<td>sd</td>
<td>2.02</td>
<td>1.51</td>
<td>0.16</td>
<td>0.82</td>
<td>0.11</td>
<td>0.015</td>
<td>0.212</td>
<td>0.54</td>
</tr>
<tr>
<td>CV</td>
<td>25.8</td>
<td>8.4</td>
<td>8.3</td>
<td>14.3</td>
<td>7.8</td>
<td>8.7</td>
<td>16.7</td>
<td>39.8</td>
</tr>
</tbody>
</table>

Results

All presented samples were harvested from the same person of the spinal column. The variation in morphometric indices in this person was rather large (Table 3.6). Also visually the figures showed many different microstructure configurations (Figures 3.11 and 3.12).

In Figure 3.11 the 110 digitally cut, central slices of three relatively homogeneous isotropic samples are shown in three successive compression steps (0%, 8%, 16% apparent strain). All three samples failed in well defined bands. Where the

Figure 3.10: Morphometry as applied to the ten subregions. The sample was subdivided into ten subregions along the anatomical axis. Bone volume fraction (BV/TV) was computed for each subregion. The lowest BV/TV value of all samples was then correlated to ultimate strength (Figure 3.13).
failure band in the sample of Figure 3.11A is almost horizontally, the failure band in the sample of Figure 3.11C is completely diagonal. Thus, in sample 3.11A the failure is mostly compressive failure, whereas the failure in sample 3.11C is mostly

Figure 3.11: Band failure in A) compression, B) compression/shear, C) shear failure mode. These samples failed in a relatively well defined band (box) whereas the other regions hardly underwent any visible or postyield deformation.
shear failure. In sample 3.11B the failure band had a relatively small angle to the cylinder axis and it can be expected that in this sample shear and compression forces determine failure. It is noteworthy that in both samples the regions outside the box did hardly undergo any visible deformation and no postyield failure is obvious.

Figure 3.12: Catastrophic failure modes. All samples failed over the whole length due to missing interconnecting trabeculae.
In Figure 3.12 the 110 digitally cut, central slices of three inhomogeneous and anisotropic samples are shown in three successive compression steps (0%, 8%, 16% apparent strain). Opposed to the samples in Figure 3.11, these structures did not fail in a well defined band but failed catastrophically over the whole sample length. The arrow in Figure 3.12A points to a relatively large, strong looking plate that completely bends during the compression cycle. It was found that there were no interconnecting horizontal trabeculae to lock in this plate. Figure 3.12B shows the subsection of a trabecular structure with relatively strong-looking vertical trabeculae and only few weak horizontal trabeculae. The two arrows point to two horizontal trabeculae that interconnect the left part of the structure to the right part. It was found that the upper of these two trabeculae was heavily bent during the compression cycle, whereas the lower broke in tension. In Figure 3.12C the arrow points to a vertical plate that is optimally oriented to absorb axial loads. We found no interconnecting horizontal trabeculae to fortify this plate, which underwent catastrophic failure during the compression cycle.

To analyze the different microstructure types in more details, the two samples from Figure 3.11B and Figure 3.12C were selected and compared visually as well as based on the morphometric indices (Table 3.7). These two samples were selected due to their similarity in BV/TV (7.71% vs. 7.49%).

The analysis of the samples with respect to the ten subregion showed that the coefficient of variation (CV) in BV/TV measured over the ten subregions ranged from 9.07% to 28.23%, thus no sample was perfectly homogeneous. When correlating the lowest BV/TV of the ten subregions to ultimate strength we found a correlation of $R^2 = 0.57$. This correlation was only marginally higher than the correlation of the BV/TV as measured for the whole sample with ultimate strength ($R^2 = 0.55$, Figure 3.13).

<table>
<thead>
<tr>
<th>sample</th>
<th>BV/TV</th>
<th>Tb.Sp</th>
<th>Tb.Th</th>
<th>SMI</th>
<th>TBPF</th>
<th>DA</th>
<th>E</th>
<th>Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[%]</td>
<td>[mm]</td>
<td>[mm]</td>
<td>[1]</td>
<td>[1/mm]</td>
<td>[1]</td>
<td>[MPa]</td>
<td>[MPa]</td>
</tr>
<tr>
<td>3.11B</td>
<td>7.71</td>
<td>1.04</td>
<td>0.146</td>
<td>2.00</td>
<td>6.92</td>
<td>1.21</td>
<td>58.2</td>
<td>1.039</td>
</tr>
<tr>
<td>3.12C</td>
<td>7.49</td>
<td>1.44</td>
<td>0.172</td>
<td>1.68</td>
<td>4.81</td>
<td>1.48</td>
<td>89.71</td>
<td>1.552</td>
</tr>
<tr>
<td>diff.</td>
<td>–3%</td>
<td>+38%</td>
<td>+18%</td>
<td>–16%</td>
<td>–30%</td>
<td>+22%</td>
<td>+54%</td>
<td>+49%</td>
</tr>
</tbody>
</table>
3.3 Limitations of morphometry

Discussion

In this study, we discuss failure mechanisms of human trabecular bone samples based on image guided failure assessment (IGFA) [10]; an extended technique to uniaxial compression testing. Based on our visual observations, we could identify samples that failed in well defined bands, whereas others failed catastrophically over the whole length. We speculated that the first failure mode primarily occurs in homogeneous and isotropic bone samples, whereas the second failure mode occurs in inhomogeneous and anisotropic bone samples. Such visual observations may help to improve our understanding of failure mechanisms which may result in new ideas to develop morphometric indices better able to catch the weaknesses of trabecular bone.

We suggest that failure of homogeneous and isotropic trabecular bone samples is determined by the weakest configuration of all possible sets of trabeculae lying on a surface that spans across the specimen. Thus, failure in such samples occurs predominantly in well defined bands, where the regions outside this band hardly undergo any deformation. This finding is supported by a study based on two-dimensional Voronoi derived finite element models done by Silva et al [39], where

![Figure 3.13: Ultimate strength versus whole BV/TV and lowest subregion BV/TV. Limiting BV/TV to the weakest subregion did not improve the correlation.](image-url)
they also reported band-like failure. Their models were extremely homogeneous and isotropic since they were generated by mathematical algorithms. In our study, we analyzed true bone samples, where a perfect homogeneity is hardly reached. Nevertheless, the samples shown in Figure 3.11 all look relatively homogeneous and isotropic. In all these samples we found a relatively well defined region where failure occurred. This failure was found in compression mode for samples 3.11A, in shear mode for sample 3.11C and in a combined compression-shear mode for sample 3.11B.

Motivated by this visual finding, we assumed that ultimate strength should better be predictable by applying morphometry on a well defined region as opposed to the whole sample. To test this idea, we correlated the lowest BV/TV of the ten subregions of each sample versus ultimate strength and compared the result with the correlation of the BV/TV of the whole sample versus ultimate strength (Figure 3.13). With this, the correlation could only marginally be increased from $R^2 = 0.55$ to $R^2 = 0.57$. There are several possible explanations for this finding. First, if all samples would be extremely homogeneous, then the BV/TV in each subregion would reflect the BV/TV of the whole sample. This was certainly not the case, since we found CV values that ranged from 9.07% to 28.23%. Thus the variability was relatively large in all samples. Second, since we found in certain specimens fracture bands that spanned diagonally through the whole sample (Figure 3.11C), the subdivision in ten equal subregions along the axis is not representative for the failure of these samples. Furthermore, the images in Figure 3.12 suggest that anisotropic samples have a tendency to fail catastrophically over the whole length. In these samples it makes no sense to divide the sample into subregions, since other mechanism such as missing interconnecting trabeculae may determine failure.

We suggest that failure of anisotropic trabecular bone samples, where the material is inhomogeneously distributed is determined by the presence and strength of interconnecting trabeculae if the load is applied along the anatomical axis. This could be demonstrated in Figure 3.12, where all samples failed over the whole length and hardly any region remained unaffected. The sample in Figure 3.12A did burst in a barreling failure mode where the plate on the right (arrow) was disrupted from the rest of the structure. This element was connected to the rest of the structure by only one very thin horizontal trabecula, where the interconnecting element was obviously not strong enough to keep the structure together and thus failed in tension. If there were more horizontal trabeculae interconnecting the strong vertical columns, catastrophic failure could eventually have been avoided. Similarly, the specimen in Figure 3.12C showed a plate (arrow) that folded completely during the compression cycle. In this sample it is very obvious from the uncompressed step that horizontal
interconnecting trabeculae were missing to support this element. The specimen in Figure 3.12B was composed of a few strong vertical columns interconnected by only a few horizontal elements (arrows). The lower arrow points to an element that was broken in tension during the failure mechanism, whereas the upper arrow points to an element that was heavily bended. It is noteworthy that both these elements interconnected the same vertical columns.

To further explore differences in samples that failed in bands as compared to samples that failed catastrophically, we selected the two samples from Figure 3.11B and Figure 3.12C, since they were similar in BV/TV (Table 3.7) but different in their failure characteristic. First, it is noteworthy, that even if both samples had almost the same BV/TV (difference = 3%), sample 3.12C was much stiffer (+54%) and also stronger (+49%) than sample 3.11B, which raises the question on how this difference can be captured. In sample 3.12C the degree of anisotropy (DA) is higher (22%) and since the bone material is mainly distributed along the anatomical axis this sample can be expected to be relatively strong when loaded along this axis, where it will probably not be very strong when loaded off axis. Opposed to this, the sample 3.11B is more isotropic and hence better adapted to off axis forces. Unfortunately, DA did not explain additional variance in ultimate strength when combined in a multiple linear regression analysis together with BV/TV. Another obvious difference was found for Tb.Sp (38%). The images even imply a larger difference in this index and indicate that the structure 3.12C fails in the area where Tb.Sp is largest. For this reason it might be interesting to assess a maximum Tb.Sp instead of the conventionally assessed mean Tb.Sp, since largely spaced areas could indicate missing interconnecting trabeculae; a potential weakness of bone microstructures. For the trabecular thickness we found a value that was 18% higher in 3.12C than in 3.11B. It has previously been suggested that vertical elements that are subjected to compression could increase in thickness as a compensatory response to loss of horizontal trabeculae [40]. Here, we had a similar situation, where in sample 3.11B many small and relatively thin trabeculae determined the strength of the structure as opposed to sample 3.12C, where the strength was determined by the main vertical columns. However, in sample 3.12C it was eventually not the large columns that determined failure but missing interconnecting trabeculae that were relatively thin. For this reason we think that Tb.Th measures are difficult in interpretation if not all trabeculae have about the same mean thickness and if the samples are not isotropic. The SMI showed a more plate-like structure type for the sample 3.12C, which was also reflected in TBPF, a measure for the mean curvature of the bone surface.
Although all samples were harvested from the spine of one person, we found a rather large variation in bone architectures and corresponding failure modes which was reflected in the images (Figure 3.11 and 3.12) as well as in the morphometric indices (Table 3.6). The apparent bone volume density ranged from 4.19% up to 12.33%, thus varied by a factor of three. For this reason it is difficult to conclude from one trabecular bone autopsy to the apparent bone density at a specific site or even at other sites within the body. The location where bone was taken from may be extremely sensitive wherefore results from autopsy and biopsy studies comparing different individuals must be taken with care. To truly investigate in person differences analysis of whole bones may yield in better results. The same limitations apply also to mechanical testing experiments of trabecular bone samples, where it is generally assumed that the samples represent a continuum. Nevertheless, many trabecular bone investigations are based on such samples, which are considered a standard nowadays.

In conclusion, we could demonstrate that two main failure mechanisms determine the competence of trabecular bone samples. Whereas in homogeneous, isotropic trabecular bone samples a whole set of trabecular elements determine the competence, in inhomogeneous, anisotropic samples a single or a missing trabeculae may be responsible for catastrophic bone failure. Since morphometric algorithms are designed to work on homogeneous trabecular bone samples where all trabeculae are of about the same type these algorithms are often not capable to capture these weaknesses and may fail in predicting bone strength under these conditions.

Acknowledgements

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Chapter 4

Idealized computational models
4.1 A finite element beam-model for efficient simulation of large-scale porous structures

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Abstract:
This paper presents a new method for the generation of a beam finite element (FE) model from a three-dimensional (3D) data set acquired by micro-computed tomography (micro-CT). This method differs from classical modeling of trabecular bone because it models a specific sample only and differs from conventional solid hexahedron element-based FE approaches in its computational efficiency. The stress-strain curve, characterizing global mechanical properties of a porous structure, could be well predicted \((R^2 = 0.92)\). Furthermore, validation of the method was achieved by comparing local displacements of element nodes with the displacements directly measured by time-lapsed imaging methods of failure, and these measures were in good agreement. The presented model is a first step in modeling specific samples for efficient strength analysis by FE modeling. We believe that with upcoming high-resolution \textit{in vivo} imaging methods, this approach could lead to a novel and accurate tool in the risk assessment for osteoporotic fractures.

Keywords:
Finite element method, Micro-compression, Bone architecture, Trabeculae, Image guided failure assessment
Introduction

Osteoporosis, which occurs most frequently in post-menopausal women and the aged, is defined as a metabolic bone disease characterized by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk [1]. It has been estimated that at least 20% of women have suffered one or more fractures by age 65, and as many as 40% suffer such fractures after the age of 65 [2]. It is increasingly recognized that osteoporosis is an important public health problem with rapidly escalating social and medical cost.

Densitometry use in the last three decades has resulted in a focus on bone density as the most important predictor of osteoporotic bone fractures for osteoporosis [3-5]. However, although many older persons may lose bone, as expressed by a decrease in bone density, not all develop fractures. This is not unexpectedly so, as bone density is not the sole determinant of fracture risk. Bone mineral density, geometry of bone, micro-architecture of bone and quality of the bone material are all components that determine bone strength as defined by the bone’s ability to withstand loading. For this reason, micro-structural information must be included in the analysis to predict individual mechanical bone properties [6,7]. Preliminary data have shown that predicting trabecular bone strength can be greatly improved by including architectural parameters in the analysis [8-10]. However, the relative importance of bone density and architecture in the etiology of bone fractures, an issue also referred to as bone “quality”, is poorly understood.

Because of the large volume fraction of vertebral trabecular bone — over 90% of the load in lumbar vertebrae is carried by trabecular bone [11,12] — an extended understanding of the failure behavior of vertebral trabecular bone is essential for estimating the risk of these spontaneous fractures. Osteoporotic fractures are typically defined as 20–25% reductions in vertebral height [13] which means that for an atraumatic fracture and the assumption that bone yields at about 1% strain [14], bone tissue is damaged long before we actually start speaking of an osteoporotic fracture. Nevertheless, most of the studies in the literature as presented above deal with the pre-yield or pre-failure behavior of bone. Mechanical tests concentrated on reporting results before the yield point or ultimate strength is reached. Only few attempts have been made to assess and quantify the mechanical behavior of bone beyond initial yielding and failure in compact [15,16] and in trabecular bone [17-19].

Pathologic anatomy may be assessed from bony fractures and relative displacements as observed in imaging studies. Recent advances in imaging methodologies
permit three-dimensional (3D) analysis of bone architecture. At the forefront of these technologies is micro-computed tomography (µCT) which is a technique for the non-destructive assessment of 3D bone architecture, and can be used for both static and pseudo-dynamic measurements. Recently, Müller et al [20] developed a method for time-lapsed measurements of micro-structural bone under compression to assess load-induced buckling and bending as well as fracture initiation and propagation in the 3D bone matrix. This method — termed image-guided failure assessment (IGFA) — allows simultaneous assessment of the 3D structure of porous materials, its global deformations, and its corresponding stress-strain values. The technique provides experimental measurements for understanding the plastic post-failure behavior of porous structures and these data can serve as the basis for the development of new theoretical models.

Many mathematical models have been proposed to investigate in the mechanical behavior of trabecular bone through representation of the microstructure as well as possible with respect to its mechanical properties. Analytical models have been widely used to represent bone mechanical behavior by exploiting generalizations in the trabecular structure. Purely analytical models have been developed, for example, using the theory of cellular materials [21-24] or a network of doubly tapered struts [25] to investigate trabecular microstructure. Others have incorporated FE analysis [26-34], and these models have been successful at identifying important failure properties of cancellous bone, for example, that bending and buckling are important failure modes. Analytic and semi-analytic models have incorporated more specific bone micro-structural properties [27-30], including the use of an open-celled matrix containing roughly spherical pores [28], anisotropic material properties with strong vertical cylindrical beam elements connected to weaker horizontal elements [27], and the inclusion of fluid representing marrow [30]. While these models have been successful for identifying important failure modes [26] and that failure occurs in bands [32], and even for models of bone remodeling [33,34], the limitation of these models is that they are generalized representations of trabecular bone.

In contrast to generalized mathematical models of trabecular microstructure, specimen specific models are also important and are essential for direct prediction of specimen bone failure properties. In this study, the focus is on development of models for specific samples for the prediction of bone strength on an individual basis. Although specimen specific models can be obtained using a solid FE [35,36] approach, such models have some practical disadvantages and limitations. These include primarily the large computational effort which can require computational times on super computers for several hours or even days and weeks [36,37]. Further-
more such models are not well suited for parametric studies where the structure can be freely altered with respect to number of elements and various element properties. Overcoming these limitations could give a deeper insight in the failure mechanism of porous structures.

We propose here a beam finite element (beam FE) model to investigate and predict failure behavior of individual trabecular bone structures. The presented beam FE model is an abstraction of the porous object (trabecular bone or a suitable substitute for research development) composed of simple cylindrical beam elements representing the trabecular elements in the real structure. While not a perfect recreation of the bone micro-architecture, its simplified representation has the advantage of an enormous decrease in the number of elements hence substantially decreasing the computational effort for those problems. Furthermore, the elements within the porous structure can be addressed and modified separately permitting parametric studies in order to gain a better general understanding of the failure behavior of trabecular bone-like structures. We believe that with upcoming high-resolution in vivo imaging methods this approach could lead to a novel and accurate tool in the risk assessment for osteoporotic fractures on a patient specific basis.

Methods

Image-Guided Failure Assessment

In order to visualize material failure on a micro-structural level, a novel micro-mechanical testing system was devised to measure unloaded and loaded porous materials directly in a micro-tomographic system (µCT) [20,38]. The micro-mechanical testing system consisted of two major components, the micro-compression device (MCD) and the material testing and data acquisition (MTDAQ) system, designed to provide uniaxial compression, data acquisition, and strain locking of the specimen during imaging. The MCD was designed to house the test specimens and act as a transportable link between the mechanical testing and CT imaging steps. Its function was to hold the specimen, lock the applied strain to the specimen, record the applied load via an onboard load-cell, and provide a radiolucent window for scanning of the specimen. The MCD has an outer diameter of 19 mm and a total length of 65 mm in order to fit in the µCT opening. The internal load chamber fits wet or dry specimens with maximal diameters of 9 mm and maximal lengths of 22 mm. The MCD in combination with µCT enables to simultaneously visualize bone failure and to assess its mechanical properties from the applied strain and the cor-
responding load. A detailed description of the used devise can be found elsewhere [38].

For the purpose of this study, a single cubic aluminum alloy (6101-T6, ERG Oakland, CA) with a side length of 6 mm and a volume density of 8% was used. The porous alloy was used because its anisotropic and inhomogeneous nature is very similar to trabecular bone found in the human lumbar spine, which has an average volume density of 9±3% [39]. The morphologic analogy between these two structures is best shown with three-dimensional visualizations (Figure 4.1). It is noteworthy that these foams are comparable to bone in terms of their morphology, but not in terms of the mechanical properties of the aluminum alloys.

For data acquisition the samples were imaged and compressed at 0, 4, 8, 16, and 20 percent nominal strain (Figure 4.2). The specimen was placed inside the MCD and preconditioned between 0.0 and 0.3% at a rate of 0.005 s\(^{-1}\) for 10 cycles to eliminate typical toe behavior [14]. Then, the specimen was transferred to the \(\mu\)CT (\(\mu\)CT 20, Scanco Medical AG, Bassersdorf, Switzerland) for initial imaging of the sample in the intact state (0%). After imaging, the specimen was returned to the MTDAQ, and exposed to a first monocyclic nominal strain of 4% in order to capture the linear and yield behavior of the material. This procedure was repeated three more times to acquire additional time-lapsed images of 8%, 16% and 20% nominal strain in the post-failure regime. The acquisition time in \(\mu\)CT for each image was

![Figure 4.1: Two typical porous structures: (a) aluminum alloy, (b) vertebral trabecular bone. Due to the high similarity of those structures aluminum alloy is used as a surrogate for human trabecular bone.](image-url)
approximately 90 minutes. From the displacement and load data acquired by the MTDAQ device a stress-strain curve was derived.

**Generation of the FE Beam Model**

The beam FE model was generated based on the reconstruction of the initial, uncompressed sample image. A three-dimensional Gaussian filtration with a sigma of 1.2 and a filter support of 1 voxel was applied to the original data to suppress noise in the image. This procedure was followed by a global segmentation technique based on the gray level histogram to separate object from background and to binarize the image. The threshold level was set to 28.6% of the image data range. The resulting binary volume served as the origin for all subsequent image processing procedures.

The next image processing step mapped each rod-like element within the structure to a beam element of the FE model using a spatial decomposition algorithm described earlier by Müller *et al.* [40]. This method capitalizes on the fact that a porous structure has increased thickness where several elements join together. For the identification of those thicker spots first, a distance transformation (DT) was applied resulting in a new image where the values of the voxels corresponded to the shortest Euclidean distance to the phase interface [41]. By thresholding this volume only the thickest spots within the structure remained in the image. The mass centers of those spots were then defined to be the nodes in the corresponding beam FE model. The connectivity describing the elements was recovered from the DT volume using a so-called “turtle” algorithm [40]. At each node a “turtle” followed the mass centers of the DT image in all possible directions searching for neighboring nodes. Once another node was found the points between the starting node and the end node were defined to be an element from the structure. A node and a connectivity list resulted from this algorithm describing the whole porous structure. This structure formed the underlying basis for a corresponding beam element FE model (Figure 4.3).

The resulting FE model was imported into the commercial FE software package.

Figure 4.2: Image sequence of an aluminum foam as acquired by the image-guided failure assessment (IGFA) technique.
Marc/Mentat (MSC Software Corporation, Los Angeles, CA) where the shape and material properties of the elements were defined. To minimize model complexity we selected all elements to have the same geometry and the same material properties. The mechanical behavior was modeled with a two step linear function. Up to the yield point at 205 MPa the material was modeled to behave linear elastically with an elastic modulus of 65.7 GPa, beyond the yield point the material was modeled to behave ideally plastic with a hardening modulus of 165 MPa. These values were chosen based on the known characteristics of the aluminum alloy.

The beam elements were connected at the nodes. At each node, no relative rotation and translation between the two connecting beams was allowed. The upper and lower platens were fully fixed to the sample, which matches the experimental conditions. In each compressive step of the simulation an analyses was performed to check whether trabeculae get in contact with the upper or lower platen. A so called “glue option” was active, indicating that once contact occurred there was no tangential motion between trabeculae and platen.

For our purpose, the best element type would be a beam with a full circular cross section. However, such an element does not exist in Marc [42]. Instead, we used an element (type 25) representing a straight, thin-walled, closed-section beam with circular cross-section. It is based on Euler-Bernoulli theory, allows for plastic deformation and includes twist, which improves the element for large displacement analyses. When assigning appropriate (“equivalent”) dimensional and material properties, this element behaves as if it were a beam with full cross-section. To test our assumptions on the behavior of this element, we performed a two step evaluation. First, a single beam composed of ten two-noded beam elements was modeled. The beam was fixed at one end, and at the other end a load was applied; for this configuration a simple analytical solution is available. In the second step we integrated the beam elements in a spatial structure. The goal of this step was to evaluate the behavior of the elements in a more realistic environment. The open cell cube model introduced by

![Figure 4.3: A spatial image decomposition algorithm enabled to map each element from the porous structure to a beam element within the FE model.](image)
Gibson [21, 43] seemed to be a good model for this purpose because of two reasons. First, this model was a good representation for our target structure, second, for this model existed an analytic solution which could be used to verify the FE simulation. The analytic solution for the relative Young’s modulus for the beam elements with quadratic cross section can be found to be [21]:

$$\frac{E^*}{E_S} = (t/l)^4$$

In this equation $E^*$ denotes the apparent Young’s modulus of the whole structure, $E_s$ denotes the Young’s modulus of the structure material, $t$ and $l$ denotes the width and the length of the beam elements respectively, which compose the structure.

**FE Analysis**

For proof of principal, one bone analogue structure was modeled and solved as described above. The boundary conditions were chosen to simulate axial compression. The bottom platen was fixed, and the top platen applied compression by displacement control in steps of 0.2%. Any nodes coming in contact with either platen due to the compression were also constrained by the platen boundaries for all subsequent steps. The FE simulation of axial compression was done up to a strain of 3%.

**Results**

**Evaluation of the beam element properties**

To test our assumptions on the use of a thin-walled beam element in representing a full circular cross-section we analyzed two configurations. In the first one, we modeled a single beam composed of ten two-noded elements. This simple model showed the expected behavior and the results were in excellent agreement with the analytic computation resulting in a deviation of the displacement at the end of the beam of less than 0.01%. In the second configuration, we used the model proposed by Gibson [21, 43] to evaluate the beam properties in a more realistic environment. In this case the relative elastic modulus $E^*/E_s$ as it was derived from the simulation was compared to the relative elastic modulus from the analytic computation for different relative densities $\rho^*/\rho$. Again, the results from our simulations were in excellent agreement with the analytic result with deviations between the two methods of less than 0.01%, indicating that this element is well-suited for our modeling purposes.
4.1 A finite element beam-model for efficient simulation

Figure 4.4: Stress-strain curves acquired by IGFA (gray) and the beam FE simulation (black). The two curves show that the beam FE simulation could predict the experimental data (IGFA) in the elastic and post yield region up to the maximal load.

Aluminum alloy

The stress-strain curve measured by IGFA has a steep slope in the first 0.8% of global strain, reaches its maximum at about 2% of global strain, and is followed by a flat plateau and a decrease in stress starting at about 5% of global strain (Figure 4.4). The mechanical behavior of the aluminum foam is therefore as expected and well described in literature [44]. The beam FE model proposed in this study was designed to predict the material behavior up to the maximum value (ultimate load). This model could therefore only predict reasonable values up to approximately 3% of global strain. However, within this range the stress-strain curve computed by the beam FE model was in good agreement with the experimental data ($R^2 = 0.92$), and these results are illustrated in Figure 4.4 showing the comparison of the experimentally measured (IGFA) and the theoretically computed (beam FE) stress-strain curves. This data illustrates that the presented model, despite its relative simplicity, is a good predictor of global failure behavior of porous structures.

Although the model was not designed to predict failure of single structural elements, the beam FE calculations of the displacements of most nodes were in good agreement with the experimental data, at least qualitatively. A typical comparison of the displacement from the beam FE model and the measurement for four representative nodes within the structure illustrates this point (Figure 4.5).

The use of a beam element model (718 elements) rather than solid hexahedron FE model (37081 elements) allowed us to reduce the number of elements by a factor of 50. With that also the average computation time could be reduced dramatically
allowing non-linear analysis with a fast response time. The computation time for all iterations in the non-linear model was less than 1 minute on a HP Unix system (PA8600, 550 MHz, 4 GB of memory).

Figure 4.5: Comparison of the displacements of four selected nodes within the structure for the beam FE model (black) and the experimental data (gray). Although the model was not designed to predict local failure mechanisms, the predicted and experimentally measured displacements were well matched at most nodes.
Discussion

In this paper we presented a new method for the generation of a beam FE model from a 3D data set acquired by micro-CT. This method differs from classical modeling of trabecular bone [21-34,43] in the fact that it models specific samples rather than generalizations, and it has the advantage over conventional solid FE approaches [35,36] to improve computational efficiency.

This model predicts global failure behavior accurately through a relatively simplified FE model simulation. The simplicity of the model has two main advantages. First, the number of elements is reduced by a factor of 50 compared to a corresponding solid FE model. The reduction of the number of elements enables the use of commercial FE solvers. The scientific FE modeling community is large and covers multiple disciplines, therefore it can be advantageous for biomedical engineers to be able to benefit from these constantly improving commercial packages rather than developing customized FE codes on a laboratory-by-laboratory basis. Additionally, the computational time to solve the problem can be reduced dramatically. Second, the simplification of the model enables to easily modify certain parameters to investigate their influences on the mechanical behavior of the structure. For example, the influence of altered connectivity in the structure can be readily investigated by removing whole elements in the beam FE model.

Even though the model was not designed to predict local failure mechanism, the displacements of selected nodes were in good agreement with the measured node displacements. Uncertainties in the prediction of the new position of a node may arise from image processing and from the fact that all elements have the same geometry and material properties. Nevertheless, this model is promising for the local prediction of failure, and this is an ongoing area of research in our laboratory.

The method has been validated analytically by modeling two simple cases, a single beam composed of ten elements and the open cellular model presented by Gibson [43], respectively. These two simple models were used since there was an analytic solution for both cases which could be compared to the FE result. The displacements as calculated by FE were in excellent agreement with the analytic results.

Although the results from the bone analogue structure were also in good agreement with the actual measurement using IGFA, there were some limiting factors affecting the accuracy of the prediction of the global failure behavior of the trabecular bone sample. One limitation was that a constant thickness of all beam elements was assumed. A second limitation was that plate-like structures have not
been modeled. These limitations present challenges for the model to be successful for predicting material behaviors of increasingly complex and inhomogeneous structures. However, these limitations can be overcome by modifying the model and accepting a tradeoff between model simplicity and accuracy. Some beneficial modifications that minimize the increase in model complexity are described below.

A first modification for a better failure prediction could be done by mapping the mean thickness of each element within the structure to a diameter of the corresponding beam in the FE model. The mean thickness can be assessed for each element by methods described elsewhere [39]. This individualization of the elements would not only allow better prediction of global failure behavior, but would possibly also enable a more reliable prediction of the displacement of single nodes and hence prediction of failure behavior of single elements. This method may allow to predict elements failing first and lead to a deeper understanding in the plastic post-failure mechanisms of porous structures.

Secondly, since the elements in the presented model were not designed to fail, the horizontal plateau and the negative slope in the stress-strain curve could not be predicted. To achieve this goal the model could be modified in a way that elements where stress exceeds a certain value change their properties or even are completely removed from the model. With this modification it might be possible to predict also the negative slope beyond the ultimate load of the stress-strain curve far beyond the yield point.

Third, in the presented model simple cylinders were used as beam elements. We propose two additional modifications to achieve a more realistic FE structure. First, a hyperbolic shape for the elements could be chosen as opposed to cylindrical. This could be approximated by mapping the inner parts of the 6 node elements to smaller radii. A modification of the elements in this sense would likely lead to an improved result since it includes the fact that nodes are thicker than the actual rods [25]. Second, a solution for plate-like structures should be found. This could be realized by introducing triangular shells representing plate-like elements. Complex plates could be subdivided into smaller triangular shells. Such a modification would allow using the model also for combined porous structures composed of plate and rod-like elements.

Conclusion

This study is a first step in developing a new model for failure prediction of porous structures. The presented model predicted a non-linear stress-strain curve that
was in good agreement with the experimental data up to ultimate load. The main advantages of the model are its simplicity resulting in a short computation time and allowing easy parametric modifications that could yield in new models with even higher predictive power. Future models could be able to simulate complex porous structures such as trabecular bone, which are composed of rod- and plate-like elements. We believe that with upcoming high-resolution in vivo imaging methods in patients this approach could lead to a novel and accurate tool in the risk assessment for osteoporotic fractures.

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References


4.1 A finite element beam-model for efficient simulation


4.2 Specimen-specific beam models for fast and accurate prediction of human trabecular bone mechanical properties

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Abstract:
Direct assessment of bone competence in vivo is not possible, hence, it is inevitable to predict it using appropriate simulation techniques. Although accurate estimates of bone competence can be obtained from micro-finite element models (μFE), it is at the expense of large computer efforts. In this study, we investigated the application of structural idealizations to represent individual trabeculae by single elements. The objective was to implement and validate this technique.

We scanned 42 human vertebral bone samples (10 mm height, 8 mm diameter) with micro-computed tomography using a 20 μm resolution. After scanning, direct mechanical testing was performed. Topological classification and dilation-based algorithms were used to identify individual rods and plates. Two FE models were created for each specimen. In the first one, each rod-like trabecula was modeled with one thickness-matched beam; each plate-like trabecula was modeled with several beams. From a simulated compression test, assuming one isotropic tissue modulus for all elements, the apparent stiffness was calculated. After reducing the voxel size to 40 μm, a second FE model was created using a standard voxel conversion technique. Again, one tissue modulus was assumed for all elements in all models, and a compression test was simulated.

Bone volume fraction ranged from 3.7% to 19.5%; Young’s moduli from 43 MPa to 649 MPa. Both models predicted measured apparent moduli equally well ($R^2 =$
0.85), and were in excellent agreement with each other ($R^2 = 0.97$). Tissue modulus was estimated at 9.0 GPa and 10.7 GPa for the beam FE and voxel FE models, respectively. On average, the beam models were solved in 219 s, reducing CPU usage up to 1150-fold as compared to 40 µm voxel FE models. Relative to 20 µm voxel models 10,000-fold reductions can be expected.

The presented beam FE model is an abstraction of the intricate real trabecular structure using simple cylindrical beam elements. Nevertheless, it enabled an accurate prediction of global mechanical properties of microstructural bone. The strong reduction in CPU time opens up ways for research that was not possible before, such as the routine assessment of mechanical properties of large bone specimens. With upcoming in vivo high-resolution imaging systems this model has the potential to become a standard for mechanical characterization of bone.

Keywords:
Bone mechanics, trabecular bone, finite element analysis, topological classification, human vertebrae

Introduction
A quantitative assessment of bone competence is essential in many basic and clinical studies, such as the understanding of failure mechanisms associated with osteoporosis, and for providing a functional characterization of bone in genetic studies; when assessed in vivo, it can provide the basis for determining the efficacy of drug treatment and identifying people at risk for bone fracture. The gold standard to determine bone competence is by assessing its mechanical properties in a functional, mechanical test. Direct mechanical testing is a straight-forward procedure, but has its limitations as it is a destructive test. Therefore, this method is not applicable in vivo. And although it can be used to test specimens in vitro, a sample can only be tested once, thereby limiting the assessment of direction-dependent characteristics. Furthermore, these tests are prone to errors, related to boundary artifacts [1,2] and to the often small size of the specimens, hampering high precision, especially for bones of small animals [3].

With the introduction of microstructural finite element (µFE) models directly generated from computer reconstructions of trabecular bone it is now possible to simulate a mechanical test in great detail and with high precision [4-6]. Additionally, these image-based models allow for the calculation of loads at the microstructural
or even the tissue level and have been used quite extensively over the last decade to accurately determine mechanical properties of bone specimens. However, these models run at the expense of large computer efforts. As a result, a routine assessment of bone mechanical properties is not possible, even when using super computers.

The introduction of model idealizations presents a way to reduce computational time; when representing each trabeculae with just one element, the number of elements can be reduced dramatically, which leads to an enormous reduction in computational efforts. We have investigated this concept before, using a trabecular bone-like aluminum structure [7]. In short, a three-dimensional representation of the specimen was based on micro-computed tomography ($\mu$CT) data. Each rod-like element within the structure was assessed using a volumetric spatial decomposition algorithm [8], and converted into a beam element in the FE model. Appropriate material properties were applied and an analysis, simulating compression until 3% apparent strain, was performed. The computationally derived force-displacement curve matched very well with the experimentally measured curve, while tremendously reducing computational time, thereby providing proof of concept for the validity of this approach.

In this study, we investigated whether the structural idealizations to represent individual trabeculae by single elements would also be applicable to human trabecular bone. Hence, whether this technique would result in strongly reduced computational times, while still providing accurate results. The objective of this study was to implement and validate this technique.

**Methods**

Our structural idealization approach consisted of modeling individual trabeculae with beams. This model was imported into a finite element (FE) package, which was used to simulate a compression test. To assess the validity of the beam FE models, we assessed its accuracy at the apparent level, hence, at the level of global elastic behavior. Accuracy was assessed in two ways: first, by comparing the results of the beam FE models relative to the directly measured elastic behavior, and second, by comparing the results relative to the results of highly detailed voxel-based FE models. All procedures and techniques are described in detail below.
**Direct mechanical testing**

Forty-two human vertebral bone samples were excised from intact spinal columns of nine donors who took part in an anatomical donation program. A specially developed drill was used to core the specimens from the lumbar and thoracic vertebrae under constant irrigation; specimen dimensions were 10 mm height and 8 mm in diameter. Preceding drilling, contact radiographs were taken from each vertebra to orient them into a position such that the main trabecular orientation was aligned with the longitudinal axis of the cored specimen. No data was available on age and disease state of the donors. The specimens were cleaned from soft tissue, embedded in PMMA to minimize end-artifacts, and after 5 preconditioning cycles, tested in compression with a speed of 0.05%/s. An external extensometer was used to measure strain over a length of 9.3 mm. The apparent Young’s modulus ($E_{\text{meas}}$) was calculated from the stress-strain curve.

**Assessment of bone microarchitecture**

Before mechanical testing, all specimens were scanned with a micro-computed tomography system ($\mu$CT40, Scanco Medical, Switzerland) using a 20 $\mu$m nominal resolution to assess the trabecular bone architecture. The reconstructed images were filtered using a constrained three-dimensional Gaussian filter to partially suppress noise in the volumes ($\sigma = 1.2$ and support = 1), and binarized using a global threshold (22.4% of maximum possible gray scale value) as previously described [9].

A cylindrical specimen with a diameter of 7 mm was digitally cut, to exclude bone fragments that might have resulted from the cutting process as well as to exclude unintentionally cut trabeculae. There was a wide range in bone volume fractions and trabecular architectures among the specimens (Figure 4.6). Bone volume fraction was calculated based on the extended marching cubes approach [10]; trabecular thickness was calculated following a direct model-independent method [11]. Component labeling was performed to remove unconnected parts of the structure. The component labeled images were spatially decomposed into rods and plates, and thickness and volume were calculated for each individual rod and plate. A short description of the volumetric spatial decomposition algorithm as well as local morphometry is given below; a detailed description can be found elsewhere [8].

The component labeled images were skeletonized using a three-dimensional homotopic, isotropic, and shape preserving thinning algorithm [12,13], yielding in a very rough two voxel thick, symmetric skeleton. Subsequently, a topology preserving algorithm called conditional erosion was used to reduce this skeleton to one voxel
thickness [8]. These skeletons are a very rough, one voxel thick representation of the original bone structure where rods with elliptic cross-section may be represented as slender plates. To overcome this problem, we applied an optimization algorithm to the skeleton to get a reasonable representation of rods and plates and to reduce surface noise. The optimized skeleton was then characterized by a slightly modified point-classification algorithm originally devised by Saha et al [14], which was able to compute for each voxel within the skeleton whether it was a surface point, a surface end point, an arc point, an arc end point, an arc-arc intersection point, an arc-surface intersection point, a surface-surface intersection point or an isolated point (Figure 4.7b). From this, the classified skeleton was spatially decomposed into rods and plates, which were expanded to their original size by applying a so-called multi-color dilation algorithm [8] (Figure 4.7c). This operation resulted in a spatially decomposed bone structure where all rods and plates were labeled with an individual number. Local morphometry was then used to compute the thickness and volume of each element within the structure [8].

Figure 4.6: Three representative specimens showing the wide range in bone volume fractions and trabecular architectures among the specimens, including rod-like (a), hybrid (b), and plate-like (c) architectures. Rods and plates were automatically identified; they are depicted in blue and red, respectively.
Beam finite element model

The spatially decomposed bone structure was used to generate a specimen-specific beam FE model. Each rod was modeled with one thickness-matched beam. The connection points as determined from the topological classification formed the nodes for each beam. Owing to the spatial complexity of large plates spanning through the whole structure the plates were modeled with several beams in a three-step approach. First, a number of so-called support nodes were distributed over the

Figure 4.7: Representative part of a specimen, showing the generation of a specimen-specific beam finite-element model. After micro-computed tomographical reconstruction (a) skeletonization and point-classification is applied (b). After multicolor dilation (c) local morphometry was used to compute the thickness and volume of each element within the structure. Element thickness and volume was assigned appropriately to the beams in the beam-FE model (d).
4.2 Specimen-specific beam models

![Diagram](image)

Figure 4.8: Measured Young’s modulus correlated well to measured bone volume fraction in a power-law regression. However, on an individual basis deviations from the regression line are large and are increasing with increasing density.

plate. Each support node was placed such that the minimum distance to the other support nodes was D; this inter-node distance D was set to 30 voxels (600 µm). Second, the support nodes were connected by beam elements, such that each node was connected to its five neighboring nodes. And third, the thickness of all beams that represent this plate was set such that their total volume equaled the volume of this plate-like trabecula (Figure 4.7d).

The resulting FE model was imported into the commercial FE software package Marc/Mentat (MSC Software Corporation, Los Angeles, CA) running on an Superdome System (Hewlett-Packard, Palo Alto, CA). For our purpose, the best element type would be a beam with a full circular cross section, allowing for geometrical and material non-linear analyses, such that future modeling of bone failure would be possible. However, such an element does not exist in Marc. Instead, we used an element (type 25) representing a straight, thin-walled, closed section beam with circular cross-section. It is based on Euler-Bernoulli theory, allows for plasticity and other material non-linearities and includes twist, which improves the element for large displacement analyses. Sixteen points are used to integrate the material behavior through the cross section. When assigning appropriate (“equivalent”) dimensional and material properties, this element behaves as if it were a beam with full cross-section. Analyses on cantilever beams showed that the calculated stresses and displacements due to axial and bending loading deviate less than 3% from the theoretical solution. Hence, introduced errors are small when using this element type,
and most likely are negligible in comparison with the most important assumption, namely that a trabecula can be modeled as a rod.

To accurately represent the experimental boundary conditions, elements representing the end-caps were added. The bottom cap was fully fixed whereas the upper cap underwent an axial displacement. The FE models were used to calculate the force needed, such that the upper platen was displaced in axial direction by 0.05 mm.

All beams in the FE model, whether representing a rod-like or a plate-like trabecula, were assigned an arbitrary tissue modulus ($E_{tissue}^{FE}$) of 10 GPa. After solving all models using Marc, the apparent modulus ($E_{app}^{beam}$) was calculated as

$$E_{app}^{beam} = \frac{\sigma_{app}}{\varepsilon_{app}} = \frac{F/A}{\Delta L/L}$$

in which $F$ is the force calculated from the FE simulation, $A$ is the specimen cross-section, $\Delta L = 0.05$ mm, and $L$ is the specimen length. The tissue level elastic modulus ($E_{tissue}^{beam}$) was calculated as:

$$E_{tissue}^{beam} = \frac{1}{n} \sum_{i=1}^{n} \left( \frac{E_{meas}^{app}}{E_{app}^{beam}} \right) E_{tissue}^{FE}$$

where $n$ represents the number of specimens. The results of the beam FE models were then scaled relative to this calculated tissue modulus.
Voxel-based finite element model

A second FE model consisting of identical hexahedral elements was created using a standard voxel conversion technique. To decrease computational time, but still ensuring accurate outcomes, the voxel size was reduced to 40 µm, which is about one fourth of the mean trabecular thickness, as recommended for numerical convergence [15]. Component labeling was performed to assure that no unconnected parts were present in the mesh. Similar boundary conditions were applied as to the beam FE model. The models were solved using the element-by-element method [16,17], which was implemented in Fortran; it ran on the same Superdome System as the commercial software that was used to solve the beam FE models. The apparent modulus \( E_{\text{app}}^{\text{voxel}} \) was calculated similar to the beam FE models. Consistently, the tissue modulus \( E_{\text{tissue}}^{\text{voxel}} \) was calculated as

\[
E_{\text{tissue}}^{\text{voxel}} = \frac{1}{n} \sum_{i=1}^{n} \left( \frac{E_{\text{meas}}^{\text{app}}}{E_{\text{app}}^{\text{voxel}}^{\text{app}}} \right) E_{\text{tissue}}^{\text{FE}}
\]

The results of the voxel-based FE models were then scaled relative to this calculated tissue modulus.
Results

The specimens covered a wide range in different micro-architectures ranging from rod-like to plate-like structures (Figure 4.6). Bone volume fraction (BV/TV) ranged from 3.7% to 19.5%; the direct measured Young’s modulus \( (E_{\text{meas}}) \) ranged from 43 MPa to 694 MPa (Figure 4.8). When using a power-law regression, BV/TV and \( E_{\text{meas}} \) correlated highly \( (R^2 = 0.88, E_{\text{app}} = 12,363 \text{(BV/TV)}^{1.79}) \). It should be noted, though, that especially for the specimens with relatively high BV/TV, large deviations were observed between measured values and the regression curve.

For the voxel FE models the number of elements is linearly related to bone volume, and ranged from 200,000 to 1,000,000 elements. The number of elements was reduced tremendously for the beam FE models, where the number of elements ranged from 3,000 to 15,000; this represents a reduction in the number of elements by a factor 73 (range: 58 to 105). Due to the nature of the two types of models, the reductions in the number of nodes is even much higher. On average, the number of nodes was reduced from 960,000 (range: 380,000 to 1,500,000) for the voxel FE models to 2,182 (range: 1,326 to 3,860) for the beam FE models, which represents a reduction by a factor of 438 (range: 240 to 618). These huge reductions in number of nodes and elements resulted in very high reductions in the CPU time to solve the models. On average, the beam FE models were solved in 219 s, while the voxel FE models took over 50,000 s to solve; hence, the beam FE models were solved 359 (range 49 to 1150) times faster than the voxel FE models (Figure 4.9). When keeping in mind that the µFE models were coarsened to 40 µm, which can be associated with an 8.5-fold increase in computational time [18], up to 10,000-fold reductions can be expected relative to 20 µm voxel models.

After solving all FE models, the tissue modulus was estimated at 9.0 GPa and 10.7 GPa for the beam FE and voxel FE models, respectively. On the apparent level, both models were in excellent agreement with each other \( (R^2 = 0.97; \text{Figure 4.10a}) \); they predicted the measured apparent modulus \( (E_{\text{meas}}^{\text{app}}) \) equally well \( (R^2 = 0.85 \text{ and } 0.86 \text{ for the beam and voxel models, respectively; Figure 4.10b}) \).

Discussion

In this study, we showed that beam FE models based on structural idealizations of trabecular bone can be used to accurately predict its mechanical properties. The importance of this work lies in the strong reduction in computational times as compared to voxel-based FE models, with over 1,000-fold reduction in CPU time. The
accuracy of the obtained results was assessed for forty-two human trabecular bone specimens in two ways: first, by comparing the results of the idealized models relative to the directly measured elastic behavior, and second, by comparing the results relative to the results of detailed voxel-based FE models. We found that the structural idealizations led to FE models in which the intricate real trabecular structure is represented by simple cylindrical beam elements. Nevertheless, it enabled an accurate prediction of global mechanical properties of microstructural bone, and its results correlated very highly with the results obtained from the voxel-based FE analyses. A few points need to be discussed.

The results of the beam FE models were in excellent agreement ($R^2 = 0.97$) with the results of the voxel-based FE models. From this we conclude that although not all micro-architectural detail is present in the beam FE models, their representation is detailed enough to accurately estimate the overall mechanical properties. Apparently, the deformation behavior of the two models is very similar. The correlation with the experimentally determined mechanical properties is somewhat less high ($R^2 = 0.85$). This is likely due to experimental error associated with direct mechanical testing [1,2]. However, it must be noted that both types of FE models only take the trabecular architecture into account. In principle, bone mechanical properties are influenced also by the local Young’s modulus, which can vary throughout the specimen, and which can vary from specimen to specimen. First attempts using parametric FE analyses [19,20] have been made to quantify these effects. Although these studies have shown that local moduli variations affect overall mechanical properties, we expect that these effects will be similar for all specimens tested in this study; furthermore, no differences in effective tissue moduli were found between osteoporotic and healthy persons [21] nor did bisphosphonate treatment influence tissue modulus [22], further emphasizing that the use of one effective tissue modulus is valid for calculating overall mechanical properties.

The calculated tissue modulus was 9.0 GPa and 10.7 GPa for the beam FE models and the voxel-based FE models, respectively. This means that the beam FE models behave stiffer than the voxel-based models. Two causes can be identified. First, the rods were modeled with one beam. In principle, the cubic displacement field normal to the axis of the beam elements should allow for proper bending. However, trabeculae that are curved in three-dimensional (3D) space, are only represented by a straight line in the beam FE model, hence, they can behave too stiff. When modeling a rod with several beams in series, the 3D nature of the trabeculae could be represented more accurately. As a results the overall stiffness of the model will reduce, and a higher tissue modulus would have been calculated. Another cause is
found in the modeling approach for the plates, which were modeled with a number of beams. To represent the 3D nature of the plates, we connected each support node with their five neighboring ones. The precise effects of the number of connections are difficult to assess because they will depend on the specific shape and size of trabeculae, and on the inter-node distance D. Although this could lead to a higher calculated tissue modulus, this is not of critical issue to the present study; the very good correlations between the outcomes of the beam FE and the voxel FE models show that their difference can be accounted for by simple linear scaling of the tissue modulus.

The great advantage of the beam-FE models is their strong reduction in CPU time which opens up ways for research that was not possible before. First, it allows for a routine assessment of bone competence. Second, we believe that this could be of great significance to perform parametric studies. Thorough analyses can now be made, where the influence of individual trabeculae can be determined by selectively deleting single, or multiple, elements. Furthermore, it allows for assessing the effects of homogeneous changes in thickness versus more localized thickness changes, i.e. only in the horizontal rods. This is a feature not possible with voxel FE models as thickness has to be changed by at least one voxel at the time. Considering that most µFE models have about four elements over the thickness of a trabeculae, adding just one element already means increasing the thickness by 25%. Third, recent work has shown that for accurate modeling of trabecular bone failure it is important not only to include material non-linearity, but also geometric non-linearity [23]. Although its principles have been studied, the analysis of bone failure for even small bone samples is currently not possible as such models are too big to solve, even on super computers. And fourth, we envision a combination of both FE techniques, where the beam FE models are solved to find appropriate boundary conditions for single trabecular elements. The local failure mechanism of these single trabecular elements can then be analyzed with highly detailed µFE models.

The highest reductions in CPU-time were found for specimens with low bone volume fraction, while more moderate reduction were found for specimens with high bone volume fraction (Figure 4.9). The reason for this finding is that the plates are modeled with relatively many elements. We expect that a further reduction in CPU time can be achieved by increasing the inter-node distance D for the plate-like trabeculae. Whether the models still provide accurate results remains to be tested.

In a recent study, Pothuaud et al [24] also used classification and subsequent beam FE analyses to calculate the stiffness of one trabecular bone sample. Although they followed a similar approach as the one in our study, the Young’s modulus
as calculated from their beam FE model underestimated the results of the voxel FE model by 80%. At this point, we can only speculate about the cause of that. The main difference between their approach and ours is the way the skeleton is obtained, in particular the way the plates are represented. Plates were represented by simple lines in the study by Pothaud et al. Visual inspection of our samples showed that most of the samples possess large plates which span the whole specimen. Representing them with one line might just be too much abstraction: as a result, the computer-representation of the specimen does not have proper mechanical integrity, hence, overall stiffness is reduced tremendously.

The experimentally determined moduli appear to be predicted equally well by the FE models ($R^2 = 0.85 - 0.86$) as by bone volume fraction (BV/TV; $R^2 = 0.87$). This may seem counterintuitive because the FE models not only intrinsically include BV/TV but also include a precise representation of trabecular architecture; this should allow for a better capability in predicting apparent Young’s modulus [25]. The high quality of fit when using BV/TV alone is likely a result of testing all samples in the principal material direction, thereby removing anisotropy effects. Furthermore, it should be noted that the relationship between BV/TV and $E_{app}^{meas}$ was fitted using an exponential function and that the variance showed heteroscedasticity, meaning that especially for the specimens with relatively high BV/TV, large deviations were observed between measured values and the regression curve. As a result, when using the calculated power law to predict Young’s modulus from BV/TV, the linear correlation with measured Young’s modulus dropped markedly, to $R^2 = 0.71$, indeed showing that the FE models possess much better predictive power.

In conclusion, we have implemented a fast, specimen-specific, beam FE model which can accurately predict global elastic properties; we have validated it against direct mechanical testing, and validated it against voxel-based $\mu$FE models. The strong reduction in CPU time opens up ways for research that was not possible before. With upcoming in vivo high-resolution imaging systems this model has the potential to become a standard for mechanical characterization of bone.

Acknowledgements

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References


4.2 Specimen-specific beam models


Chapter 5

Synthesis

The demographic changes in the last century lead to a huge increase in the elderly in most developed countries. With this, osteoporosis became a significant health problem and it was estimated that by 2050 over 6 million people will be affected by this disease [1]. As pointed out in the introduction, osteoporosis is a disease that is characterized by low bone mass and deterioration of bone structure that causes bone fragility and increases the risk of fracture. For this reason, efforts in finding independent measures predicting bone strength gained in significance over the last decades. The most obvious factor determining bone strength is bone mass, wherefore the world health organization (WHO) defined for practical reasons osteoporosis to be a value for bone mineral density or bone mineral content that was more than 2.5 standard deviations below the young adult mean value [2]. Nevertheless, since it was also recognized that bone density leaves a rather large variation in bone strength unexplained many attempts have been made to also include trabecular bone structure in its strength prediction. Consequently, many morphometric indices were devised to characterize different properties of trabecular bone. However, most of these indices derived values from trabecular bone samples as a whole and were not able to capture local information. Only few attempts have been made to also include local information as computed from individual rod and plate elements. Thus, the relative contribution of local tissue property variations to the quality and competence of trabecular bone samples is still unclear.

This thesis aimed to illuminate some of these aspects by providing a new framework for the volumetric spatial decomposition of trabecular bone structures into single rod and plate elements. With this method it became possible for the first time to analyze trabecular bone samples on an elemental basis and new indices could be derived directly from single rod and plate elements. Conventional three-dimensional morphometric algorithms were applied to single rods and plates, a method referred
to as local morphometry. This method allowed to estimate parameters such as thickness or trabecular number on a “true” local level. Additionally, also new indices could be derived such as the volume, slenderness or length of rods and plates, which may not be accessible by global methods. Nevertheless, it has to be mentioned that these local indices are sensitive to the model parameters and thus, must be interpreted in the view of these parameter settings. Furthermore, this new framework provided a basis for the development of idealized computational models for fast and accurate prediction of bone mechanical properties. The elemental information was used to design sophisticated specimen specific beam FE models, which showed a high accuracy and performed between 200 and 10'000 times faster than conventional voxel based FE models.

The major aims of Chapter 2 were to introduce the volumetric spatial decomposition method, to validate it on computer generated models, and to test the sensitivity to the model parameters. Additionally, the method was applied to a set of human trabecular bone samples to demonstrate that the algorithm worked on a large variation of different trabecular bone architectures. In this last step also the concept of local morphometry was introduced, which was used to depict anatomical site differences in this study.

Visual inspection of the decomposed bone structures showed that the identification of the elements was reasonable for analytic models as well as for trabecular bone samples. It was demonstrated that the method gave good result for all possible structure types. The elements were intuitively separated and the interfaces turned out rational and relatively smooth. Thus, with this method it was possible to extract single trabecular elements from any trabecular bone structure. This was demonstrated in a study where trabecular bone samples from five different anatomical sites were spatially decomposed into their rods and plates. It was confirmed that samples from femoral head were mainly composed of plates, samples from lumbar spine or calcaneus were mainly composed of rods, whereas samples from iliac crest showed a large variation in the relative amount of rod and plate volume.

The large variation in trabecular bone architectures reflects the high specialization and adaptation of different bones to fulfill their specific function while being as light as possible. It was suggested that the many adaptations are both, due to evolution as well as due to remodeling mechanism during lifetime [3,4]. For this reason it is difficult to conclude from the trabecular bone structure of a specific site to the trabecular bone structure of a different site. Thus, bone quality assessment and bone strength prediction based on the analysis of trabecular bone samples are only valid for the site where the sample was taken from and cannot easily be ex-
panded to the remainder of the skeleton. Since wrist, spine, and femoral head are the most common sites of osteoporotic fractures [5], risk must therefore very likely be assessed at all these sites independently.

The major aims of Chapter 3 were to use the volumetric spatial decomposition in combination with local morphometry to analyze age-related changes in trabecular bone structures, to test for age-related gender and site differences, and to relate local morphometric indices to the strength of trabecular bone samples. Furthermore, local failure mechanisms were described based on three-dimensional experimental animations of uniaxial compression tests.

It was shown that age-related changes in trabecular bone structures differed for the three sites analyzed. Lumbar spine samples were already in young persons mainly composed of rods, wherefore age-related bone loss at this site was expressed by a loss of rods. Opposed to this, femoral head samples were mainly composed of plates in young persons. Our data gave numerical evidence that age-related changes in these samples were expressed by plate perforation, followed by a transformation of plates to rods; a mechanism that was proposed in earlier studies [6,7]. Thus, age-related changes in trabecular bone structures were found to be site dependent and local morphometric indices were able to catch these differences. No significant age-related gender differences could be uncovered.

Local morphometric indices could accurately predict the stiffness of trabecular bone samples. It was found that a multiple linear correlation including the mean slenderness of rods, the percentage of bone volume occupied by rods and the mean trabecular spacing could explain over 90% in the variance of the stiffness. The advantage of this model over apparent bone volume density, which was also in good agreement with stiffness, was not only the goodness of the correlation but also that it was possible to see for the first time, what local structural changes determined the mechanical properties of the samples.

Additional to these studies including local morphometry, a further study based on animations derived from image guided failure assessment was performed to qualitatively describe local failure mechanisms in uniaxial compression testing experiments. It could be demonstrated that failure mechanisms were different in homogeneous, isotropic bone samples, where failure mostly occurred in well defined bands, as compared to inhomogeneous, anisotropic bone samples, where failure was determined by missing or weak interconnecting elements. It could further be demonstrated that uniaxial compression experiments were limited by the homogeneity assumption, which was not always met in human trabecular bone specimens.

Although it is generally accepted that osteoporotic fractures occur with a much
higher incidence in women than in men (Figure 1.3) [5,8], our results could not reveal such gender differences. The primary reason for this was that the data showed an enormous variance in all indices for all ages and for all sites. This large variation is an inherent limitation of cross-sectional studies where generally thousands of samples are needed to reveal significant trends. Compared to this, the analyzed dataset was relatively small wherefore no significant gender differences could be depicted. There are many reasons that explain the large variation in cross-sectional autopsy studies. First, the persons already differ by the fact that they died at different ages, which raises the question on why a natural died young person could not have reached a higher age. Genetic factors, sex hormones, diseases, drug therapies, smoking, excessive alcohol consumption, physical activity, nutrition, bodyweight, propensity to fall, or generally “lifestyle” are all factors that may determine the time point of death. However, all these factors also have an effect on bone quality [9-12] and it is questionable, whether the bones of a person that died at age fifty are representative for the bones of a person that died at age ninety when this person was fifty years old. These uncertainties are always inherent in cross-sectional studies and it is basically impossible to capture all of these factors. Second, it is not so clear how good a trabecular bone sample represents the site it was harvested from and furthermore the overall status of bones in the rest of the skeleton. In this thesis, it was demonstrated that the inter-individual variation in apparent bone volume density varied by a factor of three for samples taken from one single spine. Thus it is extremely difficult to take a representative bone sample. This fact is consolidated by the difficulty to identify the same anatomical location within different persons, which introduces errors in inter-individual comparisons. Furthermore, even if the exact same anatomical location could be identified in each person, it is not clear, whether the trabecular bone structure would be similarly representative for this site in each person.

Despite of all these limitations, the study dealing with age-related changes in morphometry revealed significant trends in local and global morphometric indices and it was possible for the first time to give quantitative evidence of microstructural changes on a local level. This means, that the effects in local morphometry are larger than the error introduced by the limitations of the study. Thus, local morphometry is a promising method that in future studies may reveal further structural changes on an elemental level, which possibly can be used in the development of new medications or in the assessment of individual fracture risk.

With the improvement of \textit{in vivo} imaging methods it is now possible to assess bone microstructure at extremities and with future developments it may even be
possible to assess bone architecture at any site in the body. With these methods it will be possible to perform longitudinal high-resolution studies, where some of the limitations inherent in cross-sectional studies can be overcome. Trabecular bone structures could be monitored and analyzed with local morphometry at exact the same location within the body which would allow to track “true” age-related changes. In such studies, local morphometry could have a high potential to improve the understanding of age-related bone loss on a trabecular level. However, the problems with longitudinal studies are that it is difficult to track people over many years or even decades. Furthermore, since the methods providing the highest spatial resolutions are nowadays based on X-ray images, a further problem is caused by the limitation caused by the radiation dose associated with high-resolution imaging.

Due to the limited possibilities assessing trabecular bone structure in vivo, osteoporosis and clinical fracture risk predictions are mainly based on bone density measurements. However, ex vivo the contribution of trabecular bone structure to its strength has intensely been investigated and it was demonstrated that measures such as anisotropy, mean trabecular spacing or structure type all had a potential to explain a certain amount of the variation in bone strength. In this thesis, local morphometric indices were related to trabecular bone strength and it was demonstrated that such indices could accurately predict the stiffness of trabecular bones. The relative amount of rod volume, the slenderness of the rods and their mean separation were indices that significantly predicted bone strength. Thus, it was possible to catch the local changes in trabecular bone structures that determine their strength. Nevertheless, in this thesis it was also demonstrated that global morphometric indices might fail in strength prediction if the samples violated the homogeneity assumption. This limitation also applies to the averaged local morphometric indices. These averaged values only present the characteristic of a mean element and are thus not able to catch local inhomogeneities. However, in future studies it should be possible to extract additional local information such as the relative position and hence the relative distribution of trabecular elements, which could be used to capture local inhomogeneities. The combination of such distribution information with local morphometric indices characterizing the strength of single elements could even be used to compute local morphometry tensors. It could become possible to identify the weakest configuration for each loading direction. Furthermore, such data could also give numerical evidence for the hypothesis that in plate-like structures bone competence is determined by a few relatively strong elements as opposed to rod-like structures, where the competence is determined by the relative arrangement of a whole set of relatively small elements.
Even if the mechanical properties of a trabecular bone sample could perfectly be predicted by local morphometry, there would still remain a very large uncertainty on how representative the sample was for the whole bone it was taken from. This issue becomes relevant as soon as trabecular bone samples are used to estimate a person’s bone quality and hence to predict the fracture risk. It was suggested that the smaller bone size at the end of puberty in women compared with men and a greater age-related decreases in trabecular and cortical bone mineral density explained in large part, why fragility fractures are more common in elderly women than in elderly men [13]. For this reason it will be extremely difficult if not impossible to estimate human bone strength from conventional trabecular bone samples. To achieve reliable results, whole bones must be analyzed, and it would be interesting to investigate in future studies how local morphometry performs in whole bone analysis. Furthermore, the effect of geometry could also be part of the reason why no gender differences in the trabecular bone samples could be found.

The major aim of Chapter 4 was, to use the volumetric spatial decomposition for the development of specimen specific idealized computational models. The porous structures were converted to beam finite element models, which yielded in accurate results and performed much faster as compared to conventional voxel based finite element models.

To prove the principle, in a first study one aluminum foam sample was converted to a beam-FE model, where all elements were mapped to beams of equal diameter and tissue properties. The idealized model was small enough to be solved using a commercial FE package. It could be shown that the stress-strain curve which was computed based on this model was in good agreement with the stress-strain curve as measured by uniaxial compression testing. Furthermore, visualizations illustrated that selected nodes underwent almost the same displacements in the simulation and in the experiment. Thus, it was demonstrated that this technique had the potential to reasonably compute apparent material properties.

Motivated by this finding a second study on human trabecular bone samples was devised, where a more sophisticated method was used to transform the samples to idealized beam-FE models. With this improved method individual thicknesses of rods could directly be mapped to individual thicknesses of the beams in the model. Plates were decomposed into a set of rods, which further simplified the models while maintaining the plate information. These specimen specific beam-FE models accurately predicted the apparent elastic properties of the trabecular bone samples and performed much faster (up to 10’000x) as compared to conventional voxel based FE models.
The major advantage of beam-FE models is their short computation time, which allows for different loading regimes to be computed in reasonable time. In future studies, stiffness tensors could be computed for each sample which could become a new standard measure for mechanical anisotropy. Furthermore, parameter studies on an elemental level could give new insight in the effect of specific local morphometric changes to the overall stiffness of trabecular bone samples. In such studies, material properties or thickness could be changed element wise or selected elements could even be removed from the model. Thus, the influence of specific local changes could directly be assessed in such model simulations. Moreover, such parameter studies could give numerical evidence to the hypothesis that in inhomogeneous trabecular bone samples single, relatively weak elements or elements that are even missing may initiate bone failure.

As opposed to local morphometry, the simulation of beam-FE models implicitly accounts for the spatial distribution information of the trabecular elements. The two methods thus perfectly complement one another and could be used in future studies to get an improved understanding of bone quality and its relation to mechanics. From beam-FE simulations stiffness and anisotropic strength criterions could be computed, to complement the local morphometry of the underlying elements. In this sense, stiffness and anisotropic strength criterions as computed by beam-FE models could even be considered additional local morphometric indices.

Idealized computational models as applied to autopsies or biopsies are restricted by the same sampling uncertainties as global and local morphometry. For this reason, whole bones must be analyzed to be able to reasonably predict fracture risk in osteoporotic patients. The computational efficiency of beam-FE models should make it possible to compute the mechanical properties of whole bones. With upcoming high resolution in vivo imaging techniques, this method could become a gold standard in fracture risk assessment.

In conclusion, a new framework for the volumetric spatial decomposition of trabecular bone microstructures into its basic rod and plate elements was devised and implemented. This method served as the basis for local morphometry and for the development of specimen specific idealized computational models. Both methods enabled the analysis of trabecular bone samples on an elemental level. It was possible to quantify age-related structural changes and it was also demonstrated that these changes were site specific due to the different microstructural architectures. Furthermore, the mechanical properties could well be predicted by local morphometry as well as by idealized computational models. Both methods were shown to be limited in osteoporotic fracture risk assessment if limited to biopsy samples. How-
ever, it was speculated that these limitations could be overcome by analyzing whole bones. With upcoming high-resolution *in vivo* imaging methods, these new methods may therefore become important tools for the routine assessment of fracture risk in osteoporotic patients.

### References


Appendix A

Mathematical background

This appendix provides a few mathematical definitions on digital topology, which are used to explain the thinning and point-classification algorithms. A detailed description on digital topology in three dimensions can be found elsewhere [1-4].

Consider a regular 3D lattice of points defined by triplets of coordinates \((x, y, z)\) which may take integer values. To each point a value can be assigned. If its value equals 0 it is called a background point and if its value equals 1 it is called an object point. With every point of the grid a cubic region may be associated, called voxel, which is defined by eight corners, twelve edges, and six faces that are shared with the voxels of the neighboring points. Any voxel \((i, j, k)\) has three types of neighbors, which are called 6-neighbors if they share a face \((i \pm 1, j, k), (i, j \pm 1, k), (i, j, k \pm 1)\), 18-neighbors if they share an edge \((i, j \pm 1, k \pm 1), (i \pm 1, j, k \pm 1), (i \pm 1, j \pm 1, k)\), 26-neighbors if they share a corner \((i \pm 1, j \pm 1, k \pm 1)\). If any point is a \(n\)-neighbor of an other point the two points are called \(n\)-adjacent \((n = 6, 18, 26)\). According to Saha et al [5] we use \(N(p)\) to denote the set of 27 points in the 3x3x3 neighborhood of the point \(p\) including \(p\) itself. The set of points of \(N(p)\) excluding \(p\) is denoted as \(N^*(p)\). Note that \(N^*(p)\) is the border of \(N(p)\). For a set of points \(S\) we define the 26-envelope \(E(S)\) of \(S\) as:

\[
E(S) = \bigcup_{p \in S} N(p) - S
\]

A \(n\)-path is a sequence of points \(p_0, p_1, \ldots, p_k\) such that \(p_i\) is \(n\)-adjacent to \(p_{i-1}\) for \(i = 1, \ldots, k\). An object \(X\) on the 3D lattice is called a \(n\)-connected component if for any two points in \(X\) there exists a \(n\)-path in \(X\) between these two points. The concept of defining three different types of neighbors and hence three different connectivities yields in three different topologies that can be defined for an object.
In this thesis we use the 26-connectivity for image points and 6-connectivity for background points.

A digital picture is conventionally defined as a quadruple \((V, m, n, X)\) where \(V \subset \mathbb{Z}^3\) is the 3D lattice, \(X\) the set of object points in \(V\), and \(m\) and \(n\) the topology for the object points and the background points, respectively. In this thesis, we only consider digital pictures of the form \((\mathbb{Z}^3, 26, 6, X)\). Furthermore, we define on this picture for each point \(p\) the two special neighborhood pictures \(\hat{N}(p)\) and \(\hat{N}(p)\):

\[
\hat{N}(p) = (N(p), 26, 6, (N(p) \cap X) - \{p\}) \\
\hat{N}(p) = (N(p), 26, 6, (N(p) \cap X) \cup \{p\})
\]

A structuring element \(B\) is composed of two subsets \(B^1\) and \(B^0\) of \(V\), which are both centered at the same point \(x\). \(B^1\) holds only object points, \(B^0\) only background points. \(B_X\) shall denote the set of all points such that \(B\) is included in \(X\).

Let \((\mathbb{Z}^3, 26, 6, X)\) be a digital picture. Let \(\overline{X} = \mathbb{Z}^3 - X\) be the complement of \(X\), and \(B = B^1 \cup B^0\) be a structuring element.

A morphological erosion of an image \(X\) by the structuring element \(B\) is the set \(X \ominus B\) such that all points \(B^1\) are included in \(X\):

\[
X \ominus B = \{x| B^1 \subset X\}
\]

A morphological dilation of an image \(X\) by the structuring element \(B\) is the set \(X \oplus B\) where the intersection on \(B^1\) and \(X\) is nonempty:

\[
X \oplus B = \{x| B^1 \cap X \neq \emptyset\}
\]

A morphologic Hit-Or-Miss Transformation (HMT) by the structuring element \(B\) is the set \(X \odot B\) where all points \(B^1\) are included in \(X\) and all points \(B^0\) are included in \(\overline{X}\):

\[
X \odot B = (X \ominus B^1) \cap (\overline{X} \ominus B^0)
\]

Let’s further define the Hit-Or-Miss Neighborhood Transformation (HMNT) which is defined respectively to the \(n\)-neighborhood. The HMNT of an image \(X\) by the structuring element \(B\) in the \(n\)-neighborhood is the transformation:

\[
X \odot_n B = (X \ominus B) \oplus (B_n \ominus (B^1 \cup B^0))
\]
References


Appendix B

Thinning algorithms

In this thesis, a series of algorithms was used to compute a one-voxel-thick skeleton. In a first step, a very rough two-voxel-thick skeleton was computed using the MB-3D algorithm proposed by Manzanera et al in 2D [1], 3D [2,3] and nD [4]. This algorithm is computationally fast, shape-preserving, and homotopic.

It iteratively peels off voxels from the object surface where one iteration is performed in three successive steps. In a first step all voxels that potentially can be removed are marked and stored in an image $A$. The points are found by translating the structuring elements $\alpha_i$ ($i = 1, 2, 3$) over the image in all possible orientations. If a point matches the structuring element it is stored in image $A$. In a second step all points that are needed to maintain the topology are stored in a separate image $B$. Any point where the structuring element $\beta_1$ is contained in its 18-neighborhood or the structuring element $\beta_2$ is contained in its 26-neighborhood belong to image $B$. The third step removes then all voxels from the image that belong to $A$ but not to $B$ (Table B.1). The algorithm runs until stability is reached. The five structuring elements are sketched in Figure B.1A.

In order to reduce the MB-3D skeleton to a one-voxel-thick skeleton, we introduce a new algorithm, subsequently called conditional erosion (CE-3D). It is a fully

| $A$ | $\{x|x \in X \ominus \alpha_i, i = 1, 2, 3\}$ |
| $B$ | $\{x|x \in X \ominus_{18} \beta_1 \lor x \in X \ominus_{26} \beta_2\}$ |
| $X^{n+1}$ | $X^n \setminus (A \setminus B)$ |

Table B.1: This sequence describes one iteration of the MB-3D algorithm.
parallel algorithm that can be applied to any two-voxel-thick skeleton.

To remove all dispensable points, the algorithm runs in 6 subsequent scans whereby the structuring element $\gamma_1$ is rotated in all 6 possible directions. In a first step all points that match the structuring element $\gamma_1$ are marked and stored in a separate image $C$. In a second step all points that are needed to maintain topology are stored in image $D$. Those points are detected by scanning their neighborhood for the structuring elements $\delta_i$ ($i = 1, 2$). The third step removes then all points that belong to $C$ but not to $D$ (Table B.2). This algorithm is performed only once.

The three structuring elements needed for this algorithm are shown in Figure B.1B.

Table B.2: This sequence describes one iteration of the CE-3D algorithm.

<table>
<thead>
<tr>
<th>For each of the 6 possible orientations of $\gamma$ do</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C = {x</td>
</tr>
<tr>
<td>$D = {x</td>
</tr>
<tr>
<td>$X^{n+1} = X^n \setminus (C \setminus D)$</td>
</tr>
</tbody>
</table>
References


Appendix C

Point-classification algorithm

In this thesis, a point-classification algorithm, originally devised by Saha et al was used to compute the point-type for each voxel within a skeleton (Figure C.1). This point-classification algorithm is described in details elsewhere [1], wherefore here only a short overview of the basic working principle is provided.

Let’s consider the digital picture $P = (\mathbb{Z}^3, m, n, X)$, which in this section always denotes a binary one-voxel-thick skeleton in the (26,6)-topology. Let further $\xi(p)$, $\eta(p)$, and $\delta(p)$ denote the number of object components, tunnels, and cavities in a neighborhood picture, respectively. The basic idea in determining the point-type of any given skeleton point $p \in P$, is to determine the topological changes within the image when removing this skeleton point. For this, it is sufficient to determine the topological changes in the neighborhood picture $\hat{N}(p)$ of the point $p$, which reduces the problem to determine the topological differences of $\hat{N}(p)$ and $\check{N}(p)$.

$p_1$, edge point of a surface (SE)  
$p_2$, inner point of a surface (S)  
$p_3$, junction point of surfaces (SS)  
$p_4$, junction point of surface and arcs (SC)  
$p_5$, arc end point (CE)  
$p_6$, inner point of an arc (C)  
$p_7$, junction point of arcs (CC)  
$p_8$, isolated point (I)

Figure C.1: Point-types in a surface skeleton. (adapted from [1]).
For \( \hat{N}(p) \), it can be shown that the topological properties always fulfill the following conditions. \( \forall p \in \mathcal{P} \): 

1. \( \hat{N}(p) \) contains no cavity.

2. \( \hat{N}(p) \) contains exactly one image component.

3. \( \hat{N}(p) \) contains no tunnel.

For this reason, the topological number in any neighborhood picture \( \hat{N}(p) \) equal \( \xi(p) = 1 \), \( \eta(p) = 0 \) and \( \delta(p) = 0 \). Thus, to characterize the topological differences in \( \hat{N}(p) \) and \( \hat{N}(p) \), it is sufficient to compute the topological numbers for the

<table>
<thead>
<tr>
<th>( \xi(p) )</th>
<th>( \eta(p) )</th>
<th>( \delta(p) )</th>
<th>Point type</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>( I )-type</td>
<td>( N_1 )</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>( SE )-type or ( CE )-type</td>
<td>( N_2 )</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>( C )-type</td>
<td>( N_3 )</td>
</tr>
<tr>
<td>&gt;2</td>
<td>0</td>
<td>0</td>
<td>( CC )-type</td>
<td>( N_4 )</td>
</tr>
<tr>
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<td>1</td>
<td>0</td>
<td>( S )-type or ( CC )-type</td>
<td>( N_5 )</td>
</tr>
<tr>
<td>&gt;1</td>
<td>≥1</td>
<td>0</td>
<td>( SS )-type or ( SC )-type or ( CC )-type</td>
<td>( N_6 )</td>
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<tr>
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<td>1</td>
<td>( SS )-type or ( SC )-type or ( CC )-type</td>
<td>( N_8 )</td>
</tr>
</tbody>
</table>

Table C.1: Initial decision table for the skeleton point classification. (from [1]).
After the completion of Table C.1

After the extension process

Figure C.2: Extension of SS-lines. (from [1]).

The classification based on these numbers is not unique. For this reason, further analysis in the neighborhood picture $\hat{N}(p)$ are needed, which are summarized in Table C.2. The combination of these two Tables allows for a unique classification of all skeleton points.

Nevertheless, although all points can uniquely be classified by using Tables C.1 and C.2, there are two cases, where points are badly classified, as demonstrated in Figures C.2 and C.3.

Consider first Figure C.2. All hidden point just below the vertical surface are background points. The SS-line as detected by using Tables C.1 and C.2 does not contain the two edge points, and should be extended. For this purpose, a distance function $D(p,q)$ for two points $p$ and $q$ is defined as the function

$$D(p,q) = \begin{cases} 
0 & \text{if } p = q \\
1 & \text{if } p \text{ is 6-adjacent to } q \\
2 & \text{if } p \text{ is 18-adjacent to } q \\
3 & \text{if } p \text{ is 26-adjacent to } q \\
\infty & \text{if } p \not\in N(q) 
\end{cases}$$

A point $q \in X$ in the digital picture is then called one of the nearest points of $p$, if $\forall r \in X \Rightarrow D(p,r) \geq D(p,q)$. Note, that a point $p$ can have several different nearest points. Then, the algorithm as presented in Table C.3 can be used to compute the

Figure C.3: Finding of SC-junction points. (from [1]).
Let $S_{SE}$ denote the set of all $SE$-type points in a surface skeleton. Let $p$ be an end point of a $SS$-line. Let $SS_p$ denote the set of all $SS$-type points in $N^*(p)$. Let $S_p$ denote the set of all $S$-type points in $N^*(p)$.

\[
\text{for all } q \in (N(p) - \mathcal{E}(SS_p)) \cap S_{SE} \text{ do }
\]
\[
\text{if } \xi(S_{SE} \cap N^*(q)) > 2 \text{ then }
\]
\[
q = SS - \text{type}
\]
\[
\text{else }
\]
\[
\text{if } \eta(S_{SE} \cap N^*(q)) > 0 \text{ then }
\]
\[
q = SS - \text{type}
\]
\[
\text{else }
\]
\[
\text{if } \xi(S_{SE} \cap N^*(q)) < 2 \text{ then }
\]
\[
\text{if } \exists r \in S_p \cap N^*(q) - \mathcal{E}(SS_p) \text{ such that } D(q, r) < D(q, p) \text{ then }
\]
\[
\text{select one of the nearest points of } q \in S_p \cap N^*(q) - \mathcal{E}(SS_p), \text{ say } t
\]
\[
t = SS - \text{type}
\]
\[
\text{end if}
\]
\[
\text{end if}
\]
\[
\text{end if}
\]
\[
\text{end for}
\]

Table C.3: $SS$-line extension procedure.

extension of a $SS$-line.

Consider now Figure C.3. The $SC$-type junction point can not automatically be detected by using Tables C.1 and C.2. To detect this point, Saha et al proposed the algorithm shown in Table C.4.

These two corrections finalize the classification of the skeleton-points.
Let $p$ be a $C$-type or $CC$-type point.
Let $S$ denote the set of all $S$-type, $SC$-type, and $SS$-type points in $N(p)$.
Let $S_1, \ldots, S_n$ be the 26-components of $S$.

\begin{verbatim}
for all $S_i \in S$ do
    if $S_i$ contains no $SC$-type or $SS$-type points then
        select one of the nearest points of $p$ in $S_i$, say, $t$
        $t = SC$-type
    end if
end for
\end{verbatim}

Table C.4: $SC$-type junction finding procedure.

References

Curriculum Vitae

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Born 9th October 1974 in Chur (GR), Switzerland

2005
Ph.D. thesis under the supervision of Prof. Dr. Ralph Müller and Prof. Dr. Philippe Zysset

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