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Appendix A

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Segmentation of Bone from µCT Imaging

Introduction

Micro-CT (µCT) imaging is fast becoming ubiquitous in morphometric investigations of bone. Part of the seduction of µCT imaging is the ability to represent the original sample as a computerised three-dimensional (3D) model. This may also be one of its disadvantages in that the 3D representation may appear to be an authentic representation but it may be inaccurate. If morphometric analysis is required then the investigator must have confidence that what is designated bone really is bone.

µCT imaging provides a series of two-dimensional (2D) tomographs, which enable accurate reconstruction of the specimen as a voxel-based dataset (Figure A.1 [A]). Each tomograph is equivalent to a histological section, whereby in 2D the bone matrix can be clearly delineated from marrow spaces. Importantly, the series of tomographs enables a 3D representation of the bone to be constructed. It is possible from this voxel-based dataset to measure trabecular dimensions using a sphere-fitting algorithm (7) and to apply other model-independent algorithms for bone structure, such as connectivity density (10), structural model index (8) and degree of anisotropy (10, 18). Datasets derived from µCT imaging provide a comprehensive suite of descriptive parameters for bone structure in 3D as well as measurement of the amount of bone.
A key step in the analysis of µCT datasets is the segmentation of the greyscale tomographs into a bone phase and a marrow phase and at present there are a number of available methods (1, 5, 6, 9, 14, 15) (Figure A.1 [B]). Global thresholding is the most common image segmentation method and usually involves the operator visually determining what greyscale range corresponds to bone. The greatest disadvantage with this method is that the operator usually sets the threshold value for the whole dataset, which may be up to 1000 tomographs, from a single image or only a limited number of images. It is however, impractical to manually inspect every tomograph to make allowance for grey-level differences within a large volume of bone. Automated global thresholding methods implemented in a computer program, such as Otsu’s (12), can determine a greyscale threshold value for every image in a volume.

![Figure A.1 Example tomograph from a cube of trabecular bone from the centre of the L3 vertebral body. [A] Greyscale tomograph and [B] corresponding segmented binary tomograph.](image-url)
Local image segmentation techniques are more sensitive to variability within a bone specimen in all three dimensions. The use of local image segmentation has been limited to investigators with specialised computer programming expertise and computer resources greater than that available on desktop computers (11, 16). However, as these high-end computer resources become more accessible, local image segmentation techniques will become more widely used.

Variability in global image segmentation and its effect on morphometric parameters of bone has implications for the biological interpretation of quantitative morphometric analyses. The day to day operations of a laboratory may result in multiple handlers of μCT datasets, which necessitates the establishment of standardised analytical protocols. In this study, the degree to which quantitative morphometric parameters of bone structure are affected by variability in image segmentation will be determined. Recommendations will be made to minimise measurement errors in the quantitative assessment of trabecular bone from μCT imaging.
Materials and Methods

Thoracolumbar vertebral bodies (T12, L1, L3 and L5) were obtained from a 33 year-old female cadaver at autopsy. From each vertebral body, 3 cubes of trabecular bone (10mm x 10mm x 10mm) were cut from the centre of the vertebral body using a diamond blade saw under constant water irrigation.

µCT imaging was performed on all samples (Chapter 2, Section 2.2.3). To determine a ‘gold standard’ for bone volume fraction (BV/TV) all samples were ashed (Chapter 2, Section 2.2.6) after micro-CT imaging. True bone volume fraction (BV/TV) was calculated as ash-weight (g/cm$^3$) / bone mineral material density (1.15 g/cm$^3$) (4).

Segmentation of the greyscale tomographs to discriminate bone matrix from marrow (Figure A.1) on each sample was performed manually by three operators (OP1, OP2 and OP3) using a global threshold technique in which a single grey-level threshold value was selected for each sample. Operator 1 repeated the analysis 3 months after the initial analysis (OP1$_2$). In addition, Otsu’s automatic segmentation algorithm was implemented as a custom-written routine in Matlab (The MathWorks). Otsu’s algorithm (Otsu) determines a single global grey-level threshold value for each sample (12).

CT analyser software (CTAn) provided by the manufacturer of the µCT system (Skyscan) uses the marching cubes algorithm to generate a surface rendering of the bone (Figure A.2). Using this software, the following three dimensional (3D) model-independent parameters were obtained: bone volume per total volume (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular bone pattern factor (TBPf), structural model index (SMI) and degree of anisotropy (DA) (Chapter 2, Section 2.2.3).
Figure A.2 Three dimensional reconstruction of a cube of vertebral trabecular bone imaged using μCT.

Inter-operator and intra-operator variability for all parameters were calculated as bias and random error (13). Bias is the mean of the differences between operators for the 12 samples, expressed as a percentage of the mean of one of the operators, for each parameter. Random error is standard deviation of the differences between operators for the 12 samples, expressed as a percentage of the mean of one of the operators, for each parameter. While the bias and random error may be positive or negative, only the magnitudes of the differences in morphometric parameters between operators are presented.
Results

Pooled morphometric parameters for the 12 trabecular bone samples, obtained by each operator (OP1, OP2 and OP3) using global thresholding and using Otsu’s method for thresholding (Table A.1).

Table A.1 Mean ± standard deviation of the morphometric parameters for the 12 trabecular bone samples obtained by each operator (OP1, OP2 and OP3) using global thresholding and using Otsu’s method for thresholding.

<table>
<thead>
<tr>
<th></th>
<th>OTSU</th>
<th>OP1</th>
<th>OP2</th>
<th>OP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GREY LEVEL</td>
<td>186 ± 4</td>
<td>192 ± 5</td>
<td>186 ± 2</td>
<td>179 ± 2</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>19.0 ± 1.9</td>
<td>21.5 ± 2.1</td>
<td>17.3 ± 2.2</td>
<td>19.5 ± 2.3</td>
</tr>
<tr>
<td>Tb.Th (µm)</td>
<td>183 ± 9</td>
<td>196 ± 9</td>
<td>172 ± 12</td>
<td>185 ± 11</td>
</tr>
<tr>
<td>Tb.Sp (µm)</td>
<td>728 ± 26</td>
<td>579 ± 109</td>
<td>767 ± 29</td>
<td>703 ± 48</td>
</tr>
<tr>
<td>Tb.N (mm²/mm³)</td>
<td>1.0 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>TBPf (/mm)</td>
<td>4.6 ± 0.9</td>
<td>7.4 ± 2.7</td>
<td>4.2 ± 1.2</td>
<td>4.9 ± 0.9</td>
</tr>
<tr>
<td>SMI (-)</td>
<td>1.0 ± 0.2</td>
<td>1.9 ± 0.7</td>
<td>0.9 ± 0.3</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>DA (-)</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
</tbody>
</table>

BV/TV calculated from the ash weight of the samples and BV/TV calculated from the µCT datasets, which were segmented by Otsu’s method, show excellent concordance ($r^2 = 0.91$, $p<0.0001$) (Figure A.3). Against the ‘gold standard’ ash weight method, BV/TV using Otsu’s method shows a bias of 0.2% and a random error of 1.3%.
Figure A.3 Scatter plot of BV/TV (Ashing) versus BV/TV (Ostu 3D), which shows excellent concordance between both methods ($Y = 1.2X - 2.9, r^2 = 0.91$). Broken line represents line of identity.

For grey-level threshold values, bias ranges from 0.1% to 6.9% (Table A.2) and random error ranges from 1.7% to 3.2% (Table A.3).

For BV/TV, bias ranges from 2.3% to 19.6% (Table A.2) and random error ranges from 2.6% to 9.9% (Table A.3). These errors constitute differences in individual measurements between operators in BV/TV of up to 8% (ie. For the sample taken from the right side of L5, BV/TV for OP1=21.4% and BV/TV for OP2=13.7%).

For Tb.Th, bias ranges from 1.3% to 12.2% (Table A.2) and random error ranges from 2.9% to 5.8% (Table A.3). These errors constitute differences between operators in Tb.Th of up to 35µm (ie. For the sample taken from the centre of L5, Tb.Th for OP1=192µm and Tb.Th for OP2=157µm).
For Tb.Sp, bias ranges from 3.4% to 32.4% (Table A.2) and random error ranges from 2.7% to 18.6% (Table A.3). These errors constitute differences between operators in Tb.Sp of up to 306µm (ie. For the sample taken from the right side of L1, Tb.Sp for OP1=475µm and Tb.Sp for OP2=781µm).

For Tb.N, bias ranges from 0.7% to 8.5% (Table A.2) and random error ranges from 2.1% to 5.7% (Table A.3). These errors constitute differences between operators in Tb.N of up to 0.13 mm\(^{-1}\) (ie. For the sample taken from the right side of L3, Tb.N for OP1=1.23mm\(^{-1}\) and Tb.N for OP2=1.10mm\(^{-1}\)).

For TBpf, bias ranges from 6.5% to 59.5% (Table A.2) and random error ranges from 10.1% to 51.1% (Table A.3). These errors constitute differences between operators in TBpf of up to 6.9 mm\(^{-1}\) (ie. For the sample taken from the right side of L3, TBpf for OP1=10.9mm\(^{-1}\) and TBpf for OP2=4.0mm\(^{-1}\)).

For SMI, bias ranges from 9.6% to 81.6% (Table A.2) and random error ranges from 8.5% to 67.9% (Table A.3). These errors constitute differences between operators in SMI of up to 2.1 (ie. For the sample taken from the right side of L3, SMI for OP1=2.95 and SMI for OP2=0.85).

For DA, bias ranges from 0.2% to 1.6% (Table A.2) and random error ranges from 0.8% to 1.8% (Table A.3). These errors constitute differences between operators in DA of up to 0.06 (ie. For the sample taken from the centre of T12, DA for OP1=2.01 and DA for OP2=2.07).
Table A.2 Bias between and within operators (OP1, OP2 and OP3) and Otsu’s method for all parameters.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>OP1 Vs OP1₂</td>
<td>0.8</td>
<td>2.4</td>
<td>1.3</td>
<td>6.5</td>
<td>1.2</td>
<td>11.6</td>
<td>13.5</td>
<td>0.2</td>
</tr>
<tr>
<td>OP1 Vs OP2</td>
<td>3.2</td>
<td>10.4</td>
<td>5.5</td>
<td>17.6</td>
<td>4.5</td>
<td>49.8</td>
<td>65.6</td>
<td>0.2</td>
</tr>
<tr>
<td>OP1 Vs OP3</td>
<td>3.9</td>
<td>11.2</td>
<td>7.3</td>
<td>9.1</td>
<td>4.3</td>
<td>13.7</td>
<td>18.3</td>
<td>1.4</td>
</tr>
<tr>
<td>OP2 Vs OP3</td>
<td>6.9</td>
<td>19.6</td>
<td>12.2</td>
<td>32.4</td>
<td>8.5</td>
<td>42.3</td>
<td>50.7</td>
<td>1.6</td>
</tr>
<tr>
<td>OP1 Vs Otsu</td>
<td>0.1</td>
<td>2.3</td>
<td>1.4</td>
<td>3.4</td>
<td>0.7</td>
<td>6.5</td>
<td>9.6</td>
<td>0.2</td>
</tr>
<tr>
<td>OP2 Vs Otsu</td>
<td>3.1</td>
<td>12.9</td>
<td>7.1</td>
<td>20.4</td>
<td>5.3</td>
<td>59.5</td>
<td>81.6</td>
<td>0.4</td>
</tr>
<tr>
<td>OP3 Vs Otsu</td>
<td>4.0</td>
<td>9.2</td>
<td>6.0</td>
<td>5.3</td>
<td>3.6</td>
<td>8.0</td>
<td>10.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Table A.3 Random error between and within operators (OP1, OP2 and OP3) and Otsu’s method for all parameters.

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</tr>
</thead>
<tbody>
<tr>
<td>OP1 Vs OP1₂</td>
<td>2.6</td>
<td>-2.6</td>
<td>4.5</td>
<td>17.6</td>
<td>3.7</td>
<td>32.5</td>
<td>39.2</td>
<td>1.0</td>
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<td>OP1 Vs OP2</td>
<td>2.5</td>
<td>8.7</td>
<td>4.3</td>
<td>13.2</td>
<td>4.2</td>
<td>44.6</td>
<td>57.4</td>
<td>1.2</td>
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<tr>
<td>OP1 Vs OP3</td>
<td>1.7</td>
<td>4.9</td>
<td>3.8</td>
<td>5.2</td>
<td>2.1</td>
<td>13.4</td>
<td>14.1</td>
<td>1.0</td>
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<tr>
<td>OP2 Vs OP3</td>
<td>3.2</td>
<td>9.9</td>
<td>5.8</td>
<td>18.6</td>
<td>5.7</td>
<td>32.3</td>
<td>36.9</td>
<td>1.8</td>
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<tr>
<td>OP1 Vs Otsu</td>
<td>1.9</td>
<td>4.8</td>
<td>2.9</td>
<td>5.6</td>
<td>2.1</td>
<td>10.1</td>
<td>14.5</td>
<td>0.8</td>
</tr>
<tr>
<td>OP2 Vs Otsu</td>
<td>2.8</td>
<td>8.6</td>
<td>4.4</td>
<td>14.5</td>
<td>3.7</td>
<td>51.1</td>
<td>67.9</td>
<td>1.4</td>
</tr>
<tr>
<td>OP3 Vs Otsu</td>
<td>2.4</td>
<td>5.7</td>
<td>4.0</td>
<td>2.7</td>
<td>3.3</td>
<td>11.4</td>
<td>8.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Discussion

Otsu’s method for delineating bone matrix from marrow space enables visual representations of the original bone samples to be reconstructed (Figure 3). There is excellent agreement between BV/TV calculated from images binarised by Otsu’s method and BV/TV calculated from the ashed samples (Figure 4). Morphometric analysis of the 12 bone samples (Table 1) shows that these samples consist of a network of well-connected trabecular plates with some rod-like structures (SMI = 1.0 ± 0.2) and there is a degree of preferential orientation of the trabeculae (DA = 1.9 ± 0.6).

The variability in the selection of the grey-level threshold value, as measured by bias and random error, is less than 7% between and within operators and Otsu’s method (Table 2 and Table 3). These low values indicate that all operators and Otsu’s method appear equivalent when delineating between bone matrix and marrow in the trabecular bone samples. However, these small differences constitute large differences when morphometric parameters are calculated. Specifically, up to 8% in BV/TV, 35µm in Tb.Th, 306µm in Tb.Sp and 2.1 in SMI. However, not all parameters show the same sensitivity to changes in grey-level values, such as 0.13mm⁻¹ in Tb.N and 0.06 in DA.
Increasingly, morphometric analysis of bones is used to determine the efficacy of therapeutic agents for the prevention of bone loss (2, 3). There is great interest in determining the mechanism by which particular pharmaceutical agents reduce fracture incidence in the absence of measurable changes in the amount of bone (17). The data in this study show that changing an operator during morphometric analysis can introduce errors, which can have a profound effect on biological interpretation. For example, if a pharmaceutical agent was found to increase Tb.Th by 35µm it would be assumed that a catabolic process has occurred, which would be proof of the drug’s efficacy. Also, variability of 35µm in Tb.Th within either a control group or a treated group may prevent a real difference between groups from being identified statistically.

These data serve to highlight to users of µCT imaging that subsequent morphometric analysis is highly sensitive to operating parameters. At present, there is no ‘correct’ method for segmenting bone from marrow in tomographs even though all commonly used segmentation techniques result in visually ‘correct’ 3D representations of the original bone sample. Ideally, an objective segmentation method should be used that has been validated against a reference sample. However, while objective algorithms do exist there has not been universal adoption of these techniques (14, 16).

Until universally standardised image segmentation techniques have been established it is recommended that a single user perform all morphometric analyses for a particular study. It would also be of value to investigators to perform a limited intra-operator variability study in order to have confidence that a specific user is proficient in the available techniques. All morphometric analyses will have errors associated with methodology but knowledge of the sources and magnitude of these errors will aid investigators in the determining the biological significance of their results.
References

Parallel-Plate Model

Introduction

Trabecular bone has a complex three dimensional spatial structure. Histological structural analysis based on tissue sections relies on the parallel-plate model (plate-model) to estimate 3D features. Through estimates of BV/TV and BS/TV, the plate-model enables the calculation of the trabecular architectural parameters, Tb.Th, Tb.Sp and Tb.N (6, 9, 12-14). These model based architectural parameters have been shown to correlate with model-independent measures of architecture (1, 3, 5, 7, 9, 11, 15).

Using dual energy X-ray absorptiometry (DXA), it is possible to estimate bone volume fraction in a non-invasive manner. The PMIL (Chapter 5) allows for measurement of BS/TV from projection based information. Thus, in combination, DXA and PMIL allow for the non-invasive assessment of BV/TV and BS/TV. This presents the opportunity to estimate plate-mode parameters of trabecular architecture in a non-invasive manner.

The aim of this study was to assess the ability of DXA and PMIL to measure plate-model parameters while using μCT model-independent and model-dependent measures as standards.
Materials and Methods

Vertebral bodies from 22 individuals (13 males and 9 females) with an age range 16 – 87 years and median age of 66 years were collected at post-mortem examination. In total, 58 vertebral bodies consisting of 6 T12, 6 L1, 16 L2, 16 L3, 7 L4 and 7 L5 were collected. A cube of trabecular bone was cut from the centrum of each vertebral body (Chapter 2, Section 2.1.1). Cubes were imaged by µCT and processed using standard protocols (Chapter 2, Section 2.2.2). Volumetric bone mineral density (vBMD) of cubes was measured by DXA (Chapter 2, Section 2.2.3) and the bone volume fraction (BV/TV\text{DXA}) estimated using the relationship,

$$\frac{BV}{TV} = \frac{vBMD}{\rho_{BONE}},$$

where $\rho_{BONE}$ is the density of the bone tissue and lies between 1 – 2 g/cm$^3$ (2, 4, 8, 16). A value of 1.15 g/cm$^3$ (4) was used in this study.

Standard three-dimensional (3D) model-independent measures of trabecular architecture, BV/TV$_{3D}$, BS/TV$_{3D}$, Tb.Th$_{3D}$, Tb.Sp$_{3D}$ and Tb.N$_{3D}$ were measured from the datasets (Chapter 2, Section 2.2.2). Using BV/TV$_{3D}$ and BS/TV$_{3D}$, the plate-model equivalents of the architectural parameters (Tb.Th$_{\mu\text{CT}}$, Tb.Sp$_{\mu\text{CT}}$ and Tb.N$_{\mu\text{CT}}$) were calculated (Chapter 2, Section 2.2.2). DXA based BV/TV (BV/TV$_{\text{DXA}}$) and BS/TV as measured by PMIL (Chapter 5) were used to calculate the non-invasive plate-model equivalents of architectural parameters (Tb.Th$_{\text{PMIL}}$, Tb.Sp$_{\text{PMIL}}$ and Tb.N$_{\text{PMIL}}$).
Statistical differences between group means were tested using analysis of variance (ANOVA) and Student’s t-test. Bonferroni’s post-hoc test was used to identify groups that achieved significance from ANOVA, while pairwise analyses were carried out to estimate the bias and random error between measures. Bias was defined as the mean of the difference between pair-wise measurements. Random error was defined as the standard deviation of the difference between pair-wise measurements. Regression analyses were used to test relationships between variables. All statistical analyses were performed using a combination of standard routines SPSS (SPSS Inc.) and Matlab (The Mathworks).
Results

Using the µCT 3D model-independent measures of architecture as the referent, no significant differences were observed between males and females for any measured parameter.

BV/TV of L3 trabecular bone cubes was found to be approximately 6% higher than those of L4 trabecular bone cubes (Table B.1). Both T12 and L1 trabecular bone cubes had Tb.Th₃D approximately 37µm thinner than those of L2 and L3 trabecular bone cubes (Table B.1).

Table B.1 Mean ± standard deviation of architectural parameters as measured by µCT for the various vertebral levels. Significant differences were identified using Bonferroni post-hoc analyses for BV/TVµCT between L3 and L4 (&: p = 0.04) and for Tb.Th₃D between T12 and L2 (*: p = 0.04), T12 and L3 (#: p = 0.03), L1 and L2 (%: p = 0.03) and L1 and L3 (^: p = 0.02).

<table>
<thead>
<tr>
<th></th>
<th>CV/TVµCT (%)</th>
<th>BS/TVµCT (mm²/mm³)</th>
<th>Tb.Th₃D (µm)</th>
<th>Tb.Sp₃D (µm)</th>
<th>Tb.N₃D (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T12</td>
<td>12.55 ± 3.68</td>
<td>2.36 ± 0.54</td>
<td>180 ± 15*, #</td>
<td>966 ± 132</td>
<td>0.70 ± 0.19</td>
</tr>
<tr>
<td>L1</td>
<td>12.49 ± 3.22</td>
<td>2.36 ± 0.48</td>
<td>178 ± 81%*#</td>
<td>970 ± 130</td>
<td>0.70 ± 0.18</td>
</tr>
<tr>
<td>L2</td>
<td>14.77 ± 4.36</td>
<td>2.36 ± 0.63</td>
<td>215 ± 27%*%</td>
<td>944 ± 187</td>
<td>0.70 ± 0.22</td>
</tr>
<tr>
<td>L3</td>
<td>15.10 ± 5.03*</td>
<td>2.40 ± 0.65</td>
<td>216 ± 25%*^</td>
<td>926 ± 182</td>
<td>0.70 ± 0.22</td>
</tr>
<tr>
<td>L4</td>
<td>9.53 ± 1.09*</td>
<td>1.91 ± 0.28</td>
<td>184 ± 17</td>
<td>1042 ± 132</td>
<td>0.52 ± 0.08</td>
</tr>
<tr>
<td>L5</td>
<td>11.26 ± 0.90</td>
<td>2.14 ± 0.31</td>
<td>194 ± 27</td>
<td>989 ± 129</td>
<td>0.59 ± 0.10</td>
</tr>
</tbody>
</table>
3D model-independent measures of thickness and separation were significantly (p < 0.001) larger than the thickness and separation measured using the µCT parameter based plate-model (Table B.2). Trabecular number was significantly (p < 0.001) smaller for model-independent measurement than for µCT parameter based plate-model (Table B.2).

Table B.2 Mean ± standard deviation of architectural parameters measured by model independent (µCT 3D) and µCT parameter based plate-model (µCT Plate-Model). P value indicates significance of Student’s paired t-test.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>µCT 3D</th>
<th>µCT Plate-Model</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Tb.Th (µm)</td>
<td>202 ± 27</td>
<td>115 ± 15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tb.Sp (µm)</td>
<td>961 ± 161</td>
<td>807 ± 218</td>
<td>&lt; 0.001</td>
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<tr>
<td>Tb.N (mm²/mm³)</td>
<td>0.66 ± 0.19</td>
<td>1.14 ± 0.28</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Pair-wise analyses indicated that Tb.Th and Tb.Sp were 40% and 16% smaller for the plate-model than the model-independent measurements, while Tb.N was 62% smaller in model-independent measurements (Table B.3).
The linear relationship between the architectural parameters as measured by the model-independent measurements and µCT parameter based plate-model measurements was computed (Figure B.1). Significant and strong relationships ($r^2 \in [0.78, 0.98]$) were found between all measures.

Table B.3 Results of pair-wise analyses for architectural parameters measured by model independent (µCT 3D) and µCT parameter based plate-model (µCT Plate-Model). The % column is the ratio (BIAS/Referent) x 100, where the Referent is the model-independent measure, and represents the portion of the model-independent measure that the BIAS accounts for.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BIAS (µm)</th>
<th>%</th>
<th>RANDOM ERROR</th>
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<tbody>
<tr>
<td>Tb.Th</td>
<td>86</td>
<td>43</td>
<td>16</td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>154</td>
<td>16</td>
<td>87</td>
</tr>
<tr>
<td>Tb.N</td>
<td>0.48</td>
<td>62</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Figure B.1 Linear relationships between model-independent measures and µCT parameter based plate-model measures. [A] Tb.Th_{3D} = 1.62Tb.Th_{µCT} + 14.73 (n = 58, r^2 = 0.78 and p < 0.001), [B] Tb.Sp_{3D} = 0.69Tb.Sp_{µCT} + 403.21 (n = 58, r^2 = 0.88 and p < 0.001) and [C] Tb.N_{3D} = 0.69Tb.N_{µCT} - 0.12 (n = 58, r^2 = 0.97 and p < 0.001). Dotted lines represent lines of identity.
While trabecular thickness was significantly larger in the μCT parameter based plate-model than the DXA/PMIL parameter based plate-model, trabecular separation and number were not found to be significantly different (Table B.4).

Table B.4 Mean ± standard deviation of architectural parameters measured by μCT parameter based plate-model (μCT Plate-Model) and DXA/PMIL parameter base plate-model (DXA/PMIL Plate-Model). P value indicates significance of Student’s paired t-test.

<table>
<thead>
<tr>
<th></th>
<th>DXA/PMIL Plate-Model</th>
<th>μCT Plate-Model</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tb.Th (µm)</td>
<td>55 ± 13</td>
<td>115 ± 15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tb.Sp (µm)</td>
<td>839 ± 186</td>
<td>807 ± 218</td>
<td>0.14</td>
</tr>
<tr>
<td>Tb.N (mm²/mm³)</td>
<td>1.16 ± 0.22</td>
<td>1.14 ± 0.28</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Pair-wise analyses indicated that the offset between μCT parameter based plate-model and the DXA/PMIL parameter based plate-model was less that 5% for trabecular separation and number measures (Table B.5).
The linear relationship between the architectural parameters as measured by the µCT parameter based plate-model and the DXA/PMIL parameter based model was computed (Figure B.2). While significant relationships were found between all measures, the linear relationship between trabecular thickness measures was weak ($r^2 = 0.09$). Much stronger relationships were identified between trabecular separation and number ($r^2 = 0.48$).

Table B.5 Results of pair-wise analyses for architectural parameters measured by µCT parameter based plate-model (µCT Plate-Model) and DXA/PMIL parameter based plate-model (DXA/PMIL Plate-Model). The % column is the ratio (BIAS/Referent) x 100, where the Referent is the µCT parameter based plate-model measure, and represents the portion of the µCT parameter based plate-model measure that the BIAS accounts for.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>µCT Plate-Model</th>
<th>DXA/PMIL Plate-Model</th>
<th>BIAS %</th>
<th>RANDOM ERROR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tb.Th (µm)</td>
<td>60</td>
<td>52</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Tb.Sp (µm)</td>
<td>32</td>
<td>4</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>Tb.N (mm²/mm³)</td>
<td>0.02</td>
<td>1</td>
<td>0.21</td>
<td></td>
</tr>
</tbody>
</table>
Figure B.2 Linear relationships between μCT parameter based plate-model measures and DXA/PMIL parameter based plate-model measures. [A] Tb.Th\textsubscript{μCT} = 0.34Tb.Th\textsubscript{PMIL} + 134.48 (n = 58, r\textsuperscript{2} =0.09 and p = 0.02), [B] Tb.Sp\textsubscript{μCT} = 0.80Tb.Sp\textsubscript{PMIL} + 137.46 (n = 58, r\textsuperscript{2} = 0.46 and p < 0.001) and [C] Tb.N\textsubscript{μCT} = 0.83Tb.N\textsubscript{PMIL} + 0.18 (n = 58, r\textsuperscript{2} = 0.46 and p < 0.001). Dotted lines represent line of identity.
Discussion

The parallel-plate model is a tool, which allows estimates of trabecular architectural parameters through knowledge of bone volume fraction and total bone surface. Although this model makes assumptions about the trabecular architecture (Chapter 2), several studies have shown that model based parameters correlate well with model-independent measures of trabecular architecture (7, 9-11). This implies that parallel-plate model estimates are potentially useful clinical tools. If measures of bone volume fraction and total bone surface are made in a non-invasive way, then one can estimate parallel-plate model parameters non-invasively. The aim of this study was to determine if parallel-plate model based measures of trabecular architecture could be estimated using a combination of DXA and PMIL.

Comparisons between μCT model-independent and μCT parameter based models revealed that the parallel-plate model underestimated estimates of trabecular thickness and separation, while overestimating trabecular number. This is likely attributed to deviations of the actual structure from that imposed by the parallel-plate model. Nonetheless, significant correlations were found between model-independent and model-dependent measures of trabecular architecture.

In order to allow a fair comparison, DXA/PMIL model based estimates were tested against μCT parameter based models. In these comparisons, significant relationships were identified between estimates of trabecular separation and number but not thickness. This indicates that non-invasive methodologies can estimate trabecular separation and number as effectively as μCT parameter based models. The modest nature of these relationships is likely the results of poor BV/TV estimation from vBMD measured by DXA (Chapter 7, Section 7.4). Since the relationship between PMIL based BS/TV and μCT based BS/TV is strong (Chapter 5, Section 5.1.4), one would expect that with better estimates of BV/TV, there would be significant improvements in plate-model parameters. This is simply due to the fact that the plate-model estimates of Tb.Th, Tb.Sp and Tb.N
are derived from BV/TV and BS/TV alone. No such relationships were identified for trabecular thickness.

While some differences were observed for BV/TV$_{3D}$ and Tb.Th$_{3D}$ between trabecular bone cubes from some vertebral levels, the analyses in this study were pair-wise analyses. As such, the differences found between vertebral levels were not a confounding factor in the study.

In summary, this study has demonstrated the possibility of estimating trabecular architectural measures from non-invasive modalities. While significant work has to be carried out to take such measurements into a clinical setting, this study has demonstrated the possibility and justifies further investigation.
References