Effect of specimen size on microCT cortical tissue measurements

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Aims
The increasing interest in cortical bone strength [1,2] led up to the investigation of the cortical tissue through X-ray microcomputed tomography (microCT).

In the past histology has been the gold standard for the morphometric examination of bone specimens but this new method permits the non-destructive analyses of specimens in a faster way preserving the integrity of the samples [3] and the calculation of the morphometric parameters over the whole sample’s volume.

The accuracy of microCT bone measurements can be influenced by a segmentation method that, through a threshold value, separates “bone” from “non bone” in the reconstructed grayscale datasets.

In order to validate the characterization of human cortical bone microarchitecture using micro-CT, cortical measures were compared with those obtained through histological examination [4,5]. Since studies often compare samples with a range of different sizes, the accurate measurement in specimens of unequal size is important.

The aim of this work was to compare microtomographic and histological analyses of cortical bone specimens of two different sizes, and to investigate how cortical measures are affected by errors when a different threshold value is applied.

Method
Twenty-two cortical bone samples biopsies (11 with a diameter of 3 mm (group A) and 11 with a diameter of 1.5 mm (group B)) were collected from human femurs and tibias of four Caucasian donors (age range 62-74). Samples were obtained from deceased persons without skeletal disorders. After having obtained bone biopsies from the diaphyses, all the samples were embedded in polymethylmethacrylate (PMMA), scanned by micro-CT, examined by histology and finally compared.

The bone samples were embedded in PMMA through an accurate procedure [6], and then they were acquired positioning them inside the micro-CT without the polyethylene cylinder. For the samples scanning, the device used was a Skyscan 1072 X-ray micro-CT (Skyscan, Kontich, Belgium). The cortical samples of the group A were acquired using a previously published protocol [7]: 80 KV, 125 µA, 1 mm aluminium filter, exposure time 5.9, image averaged on 2 frames, rotation 180°, rotation step 0.9°, field of view 8 x 8 mm² with a pixel size of 8 µm. The cortical samples of the group B were acquired using the following protocol: 50 KV, 200 µA, 1 mm aluminium filter, exposure time 5.9, image averaged on 2 frames, rotation 180°, rotation step 0.45°, field of view 4.16 x 4.16 mm² with a pixel size of 4.16 µm.

After the acquisition of the two groups, the cross section reconstruction was made using the software NRecon v. 1.10.1 (Skyscan), and a stack of cross-sections images was produced, with a separation of one pixel (8 µm or 4.16 µm). The same protocol was used for both the groups.

The cortical bone biopsies embedded in PMMA were later sectioned to thin slices of 100 µm, stained with light green and observed at the microscope (Leica DMR-HC, Leica Microsystems, Wetzlar, Germany). A digital image of each histological slice was taken with
Leica DC 300 camera mounted on the microscope (the final magnification was 50x, with a pixel size of 0.7 µm) and then cortical porosity, Haversian canal diameter and Haversian canal separation were determined manually.

From the stack of micro-CT cross sections, the one corresponding to the histological image was visually chosen for each sample. A rectangular-shaped ROI 1.5 x 1 mm² (183 x 136 pixels) was selected and then placed in the position that looked like the one of the histological ROI. After the selection of the ROI, a binarization of the micro-CT images was necessary in order to discriminate bone from non-bone and a threshold value had to be found. For both the procedure (ROI extraction and binarization) the software “CtAnalyzer” (Skyscan) was used.

The uniform threshold values for the acquisitions of the two groups were determined thanks to a procedure described in Perilli et al. (2007). In this way two fixed-optimal- thresholds were calculated, on for each group. Finally these thresholds were applied to the segmentation of the respective micro-CT datasets.

After having calculated the fixed thresholds, the cortical parameters were calculated using the software CtAnalyzer. For the comparison between histology and the corresponding cross section of bone sample only one histological slice for each sample was used. Cortical porosity, Haversian canal diameter and Haversian canal separation calculated on histological sections and with microCT, for the two groups were compared. Moreover, cortical parameters of group B using the threshold value obtained for the group A has been calculated in order to verify the effect of specimens size and the error in the measurements due to different thresholds.

In the comparisons for each sample were determined the actual differences in the parameters di, the percentage differences di%, the mean actual difference and the mean percentage difference for each structural parameter [8].

**Results**

A good correspondence between the micro-CT images and the histological sections was found as shown in the figure (Fig. 1).
The parameters calculated by the two techniques were highly correlated. Porosity estimated from microCT images of the group B has been plotted in fig. 2 as a function of porosity assessed from 2D histological sections. The regression had a high coefficient of determination ($R^2 = 0.97$).

![Plot of porosity estimated from microCT images of group B against porosity assessed from 2D histological sections. The regression has a high coefficient of determination ($R^2 = 0.97$).](image)

**Figure 2**

Calculating cortical parameters of group B using the threshold value obtained for the group A, the difference $d$ and the mean percentage difference $d\%$ found in the comparison are indicated in Table 1. Theregression for the porosity is in figure 3.

![Plot of porosity threshold between group B and A.](image)

**Figure 3**

"Po sample 1.5 mm, threshold group A vs group B"
### Table 1

<table>
<thead>
<tr>
<th></th>
<th>d</th>
<th>SD</th>
<th>d%</th>
<th>SD%</th>
</tr>
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<tbody>
<tr>
<td><strong>MicroCT threshold</strong></td>
<td></td>
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<tr>
<td>group A - group B:</td>
<td></td>
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<tr>
<td><strong>Porosity (%)</strong></td>
<td>0.91</td>
<td>0.25</td>
<td>14.26</td>
<td>3.76</td>
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<td><strong>Haversian canal Diameter (%)</strong></td>
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<td>1.46</td>
<td>6.29</td>
<td>3.2</td>
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<td><strong>Haversian canal Separation (%)</strong></td>
<td>-55.86</td>
<td>34.95</td>
<td>-13.46</td>
<td>7.62</td>
</tr>
</tbody>
</table>

### Conclusion

Cortical bone samples of two different group with different sizes were scanned by micro-CT after embedding in PMMA. Two optimal threshold values were found using histological sections as a reference. Comparisons with histology showed no significant differences in measurements for both micro-CT acquisitions. Although the little number of samples, this preliminary study confirmed that micro-CT analyses is a reliable method for the morphometrical characterization of cortical tissues. Moreover, these results demonstrate that, halving the diameters of cortical bone biopsies, a little difference in threshold value introduce a very small error in the porosity measurements.

### References: