Quantifying the osteocyte lacunae density in human cortical bone

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Osteocytes are the basic bone cells and are located in lacunae inside the bone matrix. Each osteocyte is connected to its neighbours through tiny channels (canaliculi) with a diameter of less than a micrometer. This osteocyte network plays an important role in mechanically induced adaptive bone remodeling, whereby the individual osteocytes work as mechano-sensors to detect states of local under- or overloading.

Strips of cortical bone obtained from the femurs of a male and female donor were cut to matchstick size (3x3x40 mm³) and scanned with the microtomography device of HZG at beamline BW2 of storage ring DORIS III with a spatial resolution of 4 micron. Both donors had been elderly people and especially the bone of the female donor appeared to be osteoporotic with larger porosity. The osteons were clearly visible as slightly darker concentric circles around the Haversian canals (Fig. 1).

Figure 1: 3D reconstruction of part of a strip of femoral bone from the male donor (3x3x27 mm³). In the top picture only the mineralized bone is displayed. In the middle picture, the non-mineralized tissues (bone marrow and blood vessels) are false-coloured in green, while in the bottom picture the mineralized bone has been removed to display the network of Haversian canals. Note that the long dimension of the sample corresponds to the longitudinal orientation of the femur.

Also the lacunae of the bone cells were seen to follow this concentric orientation. There was ample visual evidence for increased porosity in the osteoporotic bone compared to the normal bone, although this has not been quantified yet. To reduce the data-sets for three-dimensional (3-D) reconstruction, smaller cubes throughout the length of the samples were selected for reconstruction at high resolution (Fig. 2).
Figure 2: 3D reconstruction of a section (300x300x600 µm³) from the sample in Fig. 1. The picture to the left shows the bone with a false-colouring of the Haversian canals in green and the osteocyte lacunae in red, each of these shown separately in the middle and right pictures, respectively.

Using image analysis techniques on these smaller samples, their density of the osteocyte lacunae could be calculated. For the sample in Fig. 3 this was 76,692 lacunae per cubic millimeter. It can be concluded that high-resolution microtomography enables an unbiased quantification of the spatial distribution of osteocyte lacunae.

Figure 3: By selecting a sub-section from the sample in Fig. 1 and 2, it was determined that the volume fraction of the bone was 94.3 % (left). Furthermore, by manually selecting 25 osteocyte lacunae (middle, note the tiny red spots between the green sections of haversian canals) their average volume could be determined. Subsequently, the total volume of all osteocyte lacunae (right) was divided by this average volume to approximate the number of osteocyte lacunae in the sample. Finally, with the sample’s known volume, the density of the lacunae was calculated.

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