Supplementary Material 4

# Micro-CT data reconstruction

The computer hardware used for scanning and data reconstruction was based on a 64-bit Windows 10 operating system (Windows Corp., Desmond, WCA, USA) running with 128 GB main random access memory (RAM) on two XEON E5-2687 W V4 processors with 24 cores clocked to 3.00 GHz (Intel Corp., Santa Clara, CA, USA) and a Quadro M6000 graphics card with 24 GB video RAM (NVIDIA Corp., Santa Clara, CA, USA).

Prior to the reconstruction of the 2D sectional projections, the plug-in “X/Y alignment with a reference scan” in NRecon 2.0.3.7 (Bruker microCT) was applied to each scan to correct for potential movement during scanning. Furthermore, the projection data were optimized using a standard post-alignment compensation algorithm, a smoothing filter (= 1) with a Gaussian window kernel (= 2 px) to maintain the greyscale histogram range between resolutions and were also corrected for ring artefacts (= 5). In addition, beam hardening correction (= 40%) was applied to correct for X-ray intensity across the sample. To ensure greyscale comparability between all experiments, the minimum and maximum greyscale values for projection-to-image conversion were set to 0.00 and 0.22. The 16-bit TIFF image stacks resulting from reconstruction were virtually cropped to remove uninformative parts and then converted to 8-bit BMP image stacks using the 3D inspection software DataViewer 1.5.6.2 (Bruker microCT).

# Micro-CT data processing

Data processing and analysis were performed using a ThinkPad E480 system (Lenovo, Quarry Bay, Hongkong) with a 64-bit Windows 10 Pro operating system (Windows Corp.), a multi-core i5-8250U CPU with each core clocked to 1.60 GHz (Intel Corp.), 16 GB main RAM, and a UHD Graphics 620 graphic card with 32 GB shared video RAM (Intel Corp.).

Using the software CTAn 1.18.4.0+ (Bruker microCT), data processing and quantitative 3D analysis were performed based on the 16-bit TIFF image stacks. To this end, a uniform region-of-interest (ROI) surrounding each entire sample volume was selected. To extract the relevant structural features, the images had to be segmented into discrete phases by applying a minimum and maximum greyscale threshold. This step was performed in CTAn using the greyscale index histogram as well as the binary image preview in the binary selection preview tab to verify the choice by inspection. A global threshold from 26 to 255 was defined for the binary segmentation separating between pore space (black) and bone material (white). The chosen value fell into the depression between two peaks, which corresponded to cortical bone (CB) and cavities, respectively. To test its robustness, the lower grey scale value was slightly shifted about ± 5 units and the 3D analysis was run again. This procedure has been performed on four data sets with strongly differing cortical porosity (Ct.Po) values. Comparing the relative changes of the quantified parameters for - 5 units, the original value and + 5 units revealed that increasing the threshold about + 5 caused an increase between + 0.35% and + 0.5% relative to the original value of e.g. the Ct.Po, whereas lowering the threshold about 5 resulted in a relative decrease of only - 0.1% to - 0.25%. The chosen threshold is valid as the determined values of all tested samples show the same pattern. In the custom processing preview window in CTAn, a task list was created, which was then imported for every sample, thus permitting automated runs for all selected plug-ins. Before running the three-dimensional (3D) analysis, the image inside the ROI was saved as a BMP file. This enabled checking whether the rendering had been successful or whether artefacts had been introduced. 3D analysis was performed on CB canal microstructure as well as matrix. To obtain information on CB canal microstructure, the binary images were inverted so that the microstructure mimicked trabecular bone (TB) and CB cavities.

A technical issue became apparent when using the software CTAn and its plugin Batch Manager, which is designed to analyze large numbers of datasets using an automated analysis protocol. To have a control whether the rendering has been successful or whether artefacts were introduced, the image inside the ROI was saved as a BMP file for every dataset. Unfortunately, about 80% of approximately 150 analyses were erroneous. For example, often only a third of the image stack was processed or when separating between void and solid parts, the solid parts were significantly overestimated. Even slightly different data processing can lead to significant differences in the results, especially, if only small changes are expected to occur. The whole data processing procedure works well, if every step is performed manually or only one dataset was uploaded into the Batch Manager, which nullifies the time saving of this feature.