Multimodal imaging of the human knee down to the cellular level

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Abstract. Computed tomography reaches the best spatial resolution for the three-dimensional visualization of human tissues among the available nondestructive clinical imaging techniques. Nowadays, sub-millimeter voxel sizes are regularly obtained. Regarding investigations on true micrometer level, lab-based micro-CT (µCT) has become gold standard. The aim of the present study is firstly the hierarchical investigation of a human knee post mortem using hard X-ray µCT and secondly a multimodal imaging using absorption and phase contrast modes in order to investigate hard (bone) and soft (cartilage) tissues on the cellular level. After the visualization of the entire knee using a clinical CT, a hierarchical imaging study was performed using the lab-system nanotom® m. First, the entire knee was measured with a pixel length of 65 µm. The highest resolution with a pixel length of 3 µm could be achieved after extracting cylindrically shaped plugs from the femoral bones. For the visualization of the cartilage, grating-based phase contrast µCT (I13-2, Diamond Light Source) was performed. With an effective voxel size of 2.3 µm it was possible to visualize individual chondrocytes within the cartilage.

1. Introduction
The human musculoskeletal system consists of a variety of hard and soft tissue components. One of the big challenges in micro computed tomography (CT) is the simultaneous visualization of soft and hard tissue components, since the X-ray attenuation of soft and hard tissue significantly differs [1]. An optimized choice of the photon energy for bone, for example, would result in almost fully transparent cartilage. On the other hand an optimized energy for soft tissues would lead to streak artifacts due to the high absorbing hard tissues. For such specimens the use of phase tomography is of advantage [2]. During the present study at different length scale ranges the authors used a conventional CT, as currently available at radiology departments, an advanced conventional CT system, as available at the Biomaterials Science Center in Basel, and the synchrotron radiation facility Diamond in absorption and phase contrast modes. The goal is to demonstrate the performance of these CT systems in the morphological characterization of a human knee. The results allow for the proper choice of modality to characterize any other joint from mammals down to the cellular level.
2. Materials and methods

2.1. Specimen preparation and medical CT

The donated body of an 87 years old female was fixed in 10% formalin within 24 h after death following the standard protocol of the Institute of Anatomy at the University of Basel. The extraction of the knee was performed after fixation with transversal cuts about 10 cm above and below the femorotibial joint space. The extracted knee was scanned at this point using a Siemens Somatom Emotion® 16 medical CT scanner with an anisotropic voxel size of 0.3 \( \times \) 0.3 \( \times \) 0.6 mm\(^3\). For the scan the acceleration voltage of 130 kV and a beam current of 25 mA were chosen. A smooth reconstruction kernel (B31s) was chosen. For this scan, the knee was taken out of the formalin solution and measured in air. To prevent the tissue from drying out, the sample was then stored in a 4% formalin solution. During all further scans, the specimens were kept in the container with the formalin. After the first two scans the skin, fat and muscle tissues were removed. A femoral cylinder of 5 mm was extracted using a metal hollow punch from 2 cm sections for further higher resolved measurements with the laboratory CT-system. The location of the extracted cylinder was around the contact area of the femorotibial joint where a relatively thick cartilage layer was preserved.

2.2. X-ray microtomography

After medical CT scans the entire knee was measured using the nanotom® m (phoenix|x-ray, GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany). Due to the size the resulting of 65 \( \mu \)m pixel size was chosen. The scan was performed using an acceleration voltage of 180 kV and a beam current of 30 \( \mu \)A. The mean photon energy of the X-ray spectrum was increased by adding a 0.3 mm Cu-filter between source and specimen. The scan had a duration of 17 h. This data set was registered to the medical CT data set using a three-dimensional rigid algorithm with six degrees of freedom, namely three translation and three rotation degrees \cite{3}. After extraction of the femoral cylinder a high-resolution scan with a pixel length of 3 \( \mu \)m was performed. Acceleration voltage of 40 kV and a beam current of 350 \( \mu \)A were adjusted. The reconstruction of the radiographs was performed using the software phoenix datos|x 2.0.1 - RTM (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) where a cone beam filtered back-projection algorithm is used.

2.3. X-ray grating interferometry

The femoral cylinder was measured at I13-2 (Diamond Light Source, Harwell, UK) with a pixel length of 2.3 \( \mu \)m. The photon energy was set to 19 keV in order to be sensitive enough to visualize small electron density differences within the cartilage. The currently established interferometer there consisted of a beam-splitter grating \( g_1 \) with a periodicity of \( p_1 = 4.79 \) \( \mu \)m and a Si-structure height of 23 \( \mu \)m and an absorption grating having a periodicity of \( p_2 = 2.40 \) \( \mu \) and a Au-structure height of 109 \( \mu \)m. The distance between the two gratings was set to 48 cm which corresponds to the 9\(^{th}\) Talbot order. A total number of 5 phase steps were acquired with an exposure time of 5 s at each projection angle. 1600 projections were recorded over 360. During the scanning procedure, the sample was again kept in a small water tank with parallel polymethylmethacrylat plates. The data was phase-retrieved using pixel-wise Fourier analysis. The processed phase projections were then reconstructed using a modified filter kernel (Hilbert transform) in combination with standard filtered back-projection algorithm \cite{4}.

3. Results and discussion

The nanotom® m scan (Figure 1, right) shows the trabecular structure of the bone in detail and shows sharp edges between tissue borders in comparison to the medical CT (Figure 1 left). The sagittal slices show all three composing bones of the knee joint (i.e. femur, tibia and patella).
Figure 1. Saggital slices through the human knee acquired using a medical CT scanner (left) and the nanotom® m

The cartilage is visible in both medical CT and µCT data sets. However the higher resolution of the µCT data allows a much clearer discrimination of it with the background.

Figure 2 shows orthogonal slices through the femoral cylinder acquired using nanotom® m with a pixel length of 3 µm. The zoom-in image offers insights into the anatomy of the subchondral bone. The intermediate layer between bone and cartilage marked with the yellow arrows is associated with the so called mineralized cartilage zone [6]. The demarcation towards the cartilage marked with the right yellow arrows presumably represents the tide mark line. Due to their location, the bright circular structures are assumed to be calcified chondrocytes.

Figure 2. Three orthogonal slices through the femoral cylinder including a zoom-in obtained with the nanotom® m and a pixel length of 3 µm.
Figure 3. Three orthogonal slices through the femoral cylinder (also shown in Figure 2) including a zoom-in obtained using gratnig-based phase contrast at Diamond Light Source.

Although the higher resolution of the data set leads to sharp insights of the hard tissues, almost no contrast is present within the cartilage.

Grating-based phase contrast results are shown in Figure 3 by three orthogonal slices through the specimen. However, due to the low energy, which was used in order to achieve the highest possible contrast, huge artefacts are present within the bone. On the other hand the internal structures of the cartilage are visible in detail. Individual chondrocytes respectively show up inside the cartilage layer. Often more than one chondrocyte are located inside the same lagunae resulting in an ellipsoidal shape. A perpendicular orientation towards the surface in the vicinity of bone of them is present in order to gain more stiffness of the cartilage tissue In the outer region of the cartilage they are generally smaller and rather oriented parallel to the surface.

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