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## Hard X-ray index of refraction tomography of a whole rabbit knee joint: A feasibility study

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## ABSTRACT

We report results of the computed tomography reconstruction of the index of refraction in a whole rabbit knee joint examined at the photon energy of 51 keV. Refraction based images make it possible to delineate the bone, cartilage, and soft tissues without adjusting the contrast window width and level. Density variations, which are related to tissue composition and are not visible in absorption X-ray images, are detected in the obtained refraction based images. We discuss why refraction-based images provide better detectability of low contrast features than absorption images.

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## 1. Introduction

X-ray absorption computed tomography (ACT) performed with synchrotron radiation sources and monochromatic beam is sensitive to very small differences in the attenuation between adjacent volume elements and offers very high spatial resolution. However frequently sufficient contrast can be achieved only at the expense of large dose deposited in the sample. X-ray phase contrast imaging methods (XPCI) offer better sensitivity to fluctuations of the electron density and can be used for imaging of objects that exhibit almost no absorption-based contrast at higher X-ray energies. As imaging at higher energies often allows reducing the dose deposited in samples, XPCI methods are considered as very promising for biomedical research and imaging applications. Nonetheless in the majority of studies XPCI was applied for examination of small samples with projected thickness in the mm-cm range. The primary reasons for this limitation are the necessity of complicated instrumentation and optical elements and, in part, the lack of efficient reconstruction methods. For instance, it is very difficult to take into account in the reconstruction and accurately measure the differential phase signal at sharp interfaces (f.i. between a bone

and soft tissue or a sample container and air) or near an apex, where X-ray refraction angles are large and total reflection can occur.

Recently more realistic, thick and complex samples (dimensions up to 15 cm) have been examined within our and several other teams. In some cases homogeneous and low-absorbing specimens could be quantitatively investigated by using the propagation based phase contrast imaging method [1]. Feasibility of this approach for imaging of larger phantoms and whole human breast samples was studied [2,3]. However, this approach fails when samples contain bone or, in general, features with very different electron densities (f.i. a metal inclusion in a plastic [4]); in these cases, the assumption of object homogeneity and the phase-attenuation duality approximation [5] are no more satisfied. Differential phase-contrast imaging with grating interferometry [4], analyzer crystal [6], or coded apertures method [7] offers an alternative solution for phase retrieval, which is not limited by the phase-attenuation duality constrain. These methods can be used for quantitative phase imaging and the accurate reconstruction of the refractive index in samples, which contain even bone, calcified tissues, or metal parts [4,8]. Note that since phase delays are unambiguously related to materials' refractive index, notions of quantitative phase contrast imaging and index of refraction tomography are both used in the literature.

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CT imaging of bone and cartilage tissue in the human and animal joints is very important for detection, grading, and studies of osteoarthritic disease (OA). Whilst it is desirable to depict fine structure of the bone and surrounding soft tissues simultaneously, it is difficult to achieve it with existing techniques. Conventional X-ray ACT does not allow distinguishing cartilage layer near the bone surface – only the change of distance between bones, indirect OA sign, can be detected. Magnetic resonance imaging typically provides a very high contrast for all soft tissues, however bony tissue is poorly visualized on these images. At synchrotron, a high spatial resolution, monochromatic propagation-based XPCI was used for tomographic imaging of cartilage within a whole large human knee joint [9]. It was shown that propagation based phase-contrast images provide better visibility of cartilage tissue than ACT. In this work a sophisticated image processing and segmentation were used in order to delineate the bone. An optimized gray level windowing was then applied in order to achieve sufficient contrast of the soft tissues. Another remarkable study was performed using a laboratory X-ray source and grating interferometry setup [8]. Substantial improvement in visibility of cartilage layers close to intervertebral discs in a human cervical spine was achieved. However, contrast in obtained CT images does not allow the detection of density gradients in the soft tissue, such as cartilage, skin, ligaments or tendons. As stated by authors, current performance of a grating interferometer setup combined with a broad, polychromatic source is rather limited.

In this work we employ the analyzer crystal based phase contrast imaging technique [10] for microtomography of whole and intact rabbit knee joint. This XPCI method allows accurate measurements of differential phase contrast signal, which is essential for quantitative refraction-based CT (RCT) reconstruction. It is found that not only visualization of bone, cartilage, and soft tissues is possible, but also fine density gradients in tissues can be seen. However spatial resolution of images does not allow detection of very small structural changes in the cartilage tissue and adjoining bone, which are the first manifestation of tissues' damage and OA.

## 2. Material and methods

Differential phase images of specimens were acquired at the biomedical beamline ID17 of European Synchrotron Radiation Facility using the analyzer crystal setup and monochromatic photon beam with a mean energy of 51 keV. The so-called in-plane acquisition geometry [11] was used, which means that the differential phase signal depends on the amount of X-ray deflection in the CT reconstruction plane. Phase-delay projections were calculated from differential phase images by solving the regularized phase retrieval equation [11]. This approach was selected because the regularization term can be used to reduce artifacts arising at the bone surface, which are particularly strong in imaging with high spatial resolution. Ordinary filtered backprojection algorithm was used then for CT reconstruction.

Intact formalin fixed rabbit legs were placed in cylindrical plastic containers with an inner diameter of 50 mm. The residual volume in the containers was filled in with solidified agarose gel. The detection system composed of Gd-based scintillator couple with a CCD camera provided an effective voxel size of  $46 \mu\text{m}^3$ . The choice of 51 keV photons is explained by the enhanced absorption of the scintillator screen at this energy, which allowed us to reduce the exposure time and the dose delivered to our samples. Both absorption and refraction based data sets contained 1000 raw projections. For RCT, pairs of raw phase contrast images taken at two positions of the analyzer crystal were combined in order to obtain 500 differential phase projections (detailed description of setup parameters and acquisition sequence can be found in

reference [6]). Therefore twice less projections were used for the reconstruction of refraction based images. The photon flux was  $(9.72 \pm 0.04) \times 10^8$  and  $(3.10 \pm 0.02) \times 10^{10}$   $\text{ph}/\text{mm}^2 \cdot \text{s}$  for RCT and ACT acquisitions, respectively. In these conditions the total acquisition time was about 10 min for the ACT data set and one hour for the RCT data set. Both CT data sets were acquired at comparable doses, since the difference in the photon flux was balanced by the longer exposure time. The long acquisition time is explained by a small vertical size of the X-ray beam ( $\leq 2$  mm) and the necessity of scanning the sample. This is a general problem of analyzer crystal based imaging in the Bragg (reflection) geometry. This limitation can be potentially overcome by working in the Laue (transmission) geometry [12].

## 3. Results

The CT reconstruction of the linear attenuation coefficient  $\mu$  and index of refraction decrement  $\delta$  (imaginary and real parts of the refractive index  $n = 1 - \delta + i \times \mu/2k$  respectively, where  $k$  is the wavenumber) is presented in Fig. 1. Axial and sagittal planes are shown in the left and right columns correspondingly. Fig. 1a, b and e, f are shown in the full contrast range to present both bony and soft tissue. Bone dominates the contrast in ACT images, so that very few details can be distinguished in the soft tissues especially if the contrast inside the bone does not reach saturation. In the case of saturated contrast in the bone, the inner differences inside the bone layers are not visible. In order to enhance the visibility of soft tissues, a narrow contrast window centered at lower values of  $\mu$  was applied (Fig. 1c and d). It can be seen that even if the contrast of the absorption CT image is enhanced, only a very small improvement over the original image can be obtained. At the same time, the index of refraction image allows distinguishing details in both the soft and bony tissues. Insets in each panel show magnified regions close to the bone surface. Arrows in inset Fig. 1g indicate visible borders of cartilage layers. Perceptible variations of gray shades in the same region can be related to changes in cartilage density, although this suggestion is yet to be confirmed with histology. In addition, a change in the density of the bone's inner part and surface are also visible. Thus, refraction based images can be potentially used to examine the impact of osteoarthritis on both the cartilage and the bone. It can be argued that sufficient contrast in soft tissues can be achieved at lower photon energy. However, a simple estimation can show that the absorbed dose is 2–2.5 times smaller when imaging at 51 keV energy than at 30 keV (basing on the ratio of the mass-energy absorption coefficients and the respective photon energies).

## 4. Discussion

The different dependence on the atomic number  $Z$  between ACT and RCT is one of the reasons for better visibility of soft tissues in RCT images. It is normally being stressed that the different dependence on energy of  $\delta(E)$  and  $\mu(E)$  is the distinguishing feature of the XPCI since it means that at higher energies phase interaction mechanism is stronger than attenuation [13]. However, large values of phase delays, experienced by the wave upon propagation in a sample, are not a sufficient requirement for a good image contrast. The relative difference between reconstructed values is what enables the observer to distinguish different tissues and features in an image, providing that the signal to noise ratio is sufficiently high as well. Indeed in recent systematic studies of quantitative XPCI [4,8] it was pointed out that at higher energies, when Compton scattering becomes the dominating process for materials with low effective atomic numbers ( $Z \leq 8$ ), the signal of both modalities – attenuation and phase contrast – is proportional to the electron

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