EUROPEAN SYNCHROTRON RADIATION FACILITY

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Experiment Report Form

	Experiment title: Nano-Imaging of Osteoarthritis in Cartilage	**Experiment number**: MD1063
Beamline: ID16a	**Date of experiment**: from: 26/04/17 to: 29/04/17	**Date of report**: 31/07/17
Shifts: 9	**Local contact(s)**: Yang Yang	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Johannes Stroebel, Faculty of Physics, LMU, Munich, Germany

* Prof. Dr. Coan Paola, Faculty Physics and Faculty Medicine, LMU, Munich, Germany

Dr. med. Annie Horng, Institute for Clinical Radiology, LMU Hospital, Munich, Germany
Dr. med. Tobias Geith, Institute for Clinical Radiology, LMU Hospital, Munich, Germany

The aim of experiment

The objective of the study is to perform nano-holotomography of human cartilage for a better understanding of the anatomical and physiological factors associated with osteoarthritis (OA). We want to visualize the basic components of the articular cartilage and their arrangement within the tissue in healthy and osteoarthritic specimens extracted from the human knee. The changes induced by OA (involving, for instance, the orientation of collagen fibers, different clustering of the chondrocyte cells and concentration of proteoglycans) will be analyzed.

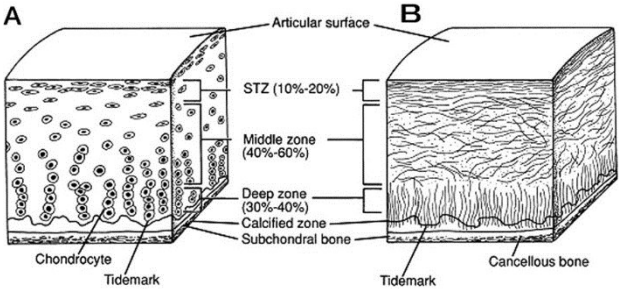

Figure 1: *The three zones of healthy articular cartilage (deep-radial zone, middle-transizional zone, and superficial-tangential zone), including orientation and arrangement of collagen fibers and chondrocytes.*
(http://sph.sagepub.com/content/1/6/461/F2.expansion.html)

In contrast to conventional histology, where the tissue has to be cut into thin slices, nano-tomography offers a continuous three-dimensional depiction of the complex network of collagen, chondrocytes, and calcifications without breakdown of the tissue.
We want to quantify the orientation of the collagen fibrils and their three-dimensional changes during degeneration providing yet unknown information about the exact initiation of degradation. This might clarify the connections between the constituents of articular cartilage, biomechanics, and function and provide a base for developing strategies against early degeneration.

Experiment and preliminary Results

We investigated 6 human osteoarthritic osteochondral plugs, i.e. portions of bone-cartilage tissue extracted from a human knee and provided by the Forensic Medicine Department of the LMU hospital following the regulations of the Ethical Committee of the LMU. Two samples were non-degenerated (healthy cartilage) and four of them were specimens with visual signs of mild OA. Two of them were harvest from the patellar cartilage where as two were from the femoral cartilage. Samples were formalin fixated, embedded in paraffin and cut in cylinders of about 500 microns in diameter and 3 mm in length.
We applied X-ray nano-holotomography using 17 keV X-rays. We imaged, using a voxel size of 100^3 (field of view 200 μm), three different regions along the height of the cartilage tissue (one region per cartilage zone).

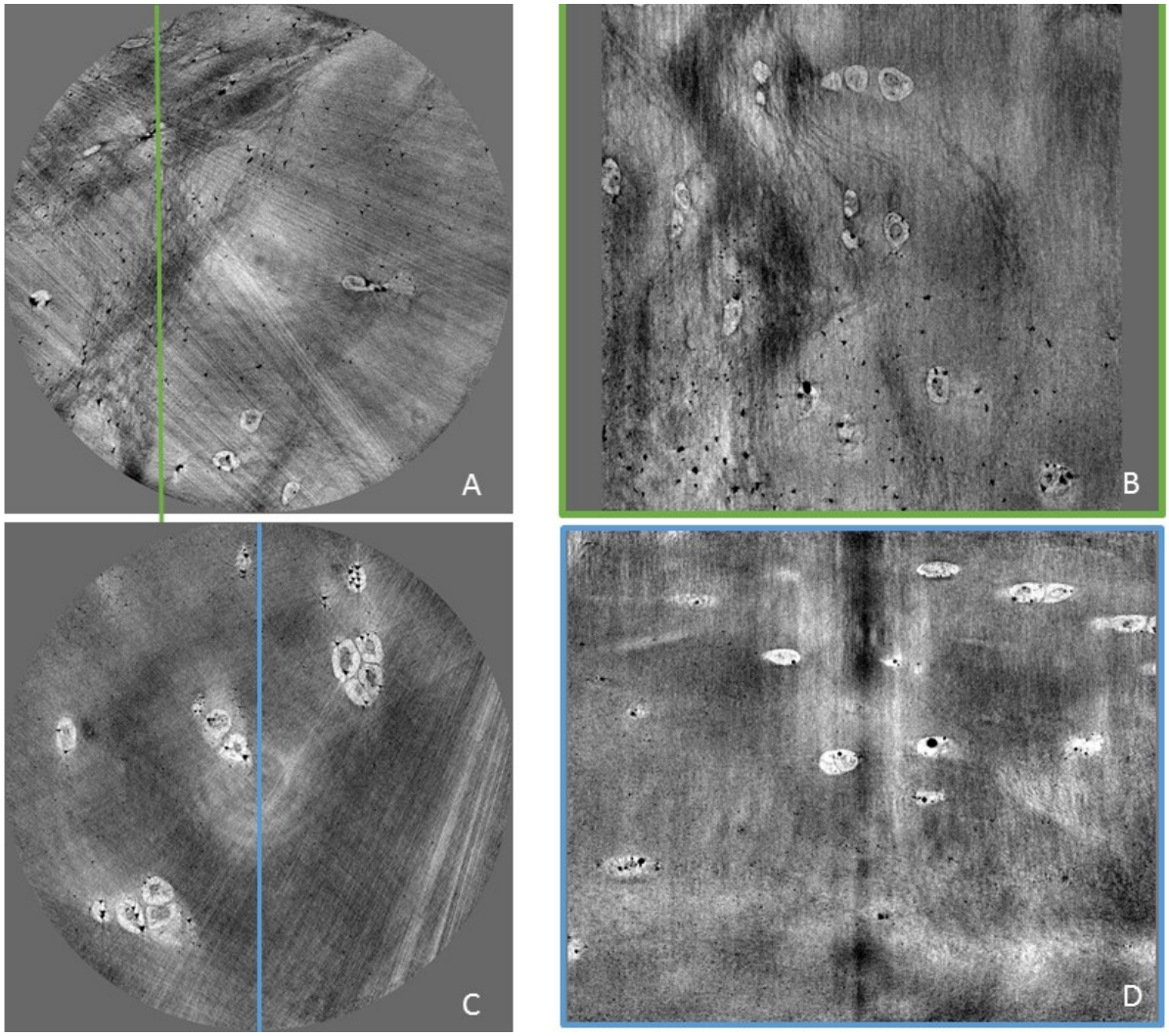

Figure 2: X –Ray holotomography images of the superficial-tangential zone in degenerated (top) and non-degenerated (bottom) cartilage. Left: axial view of the 3D reconstructed volume; right: sagittal view along the line in the axial view

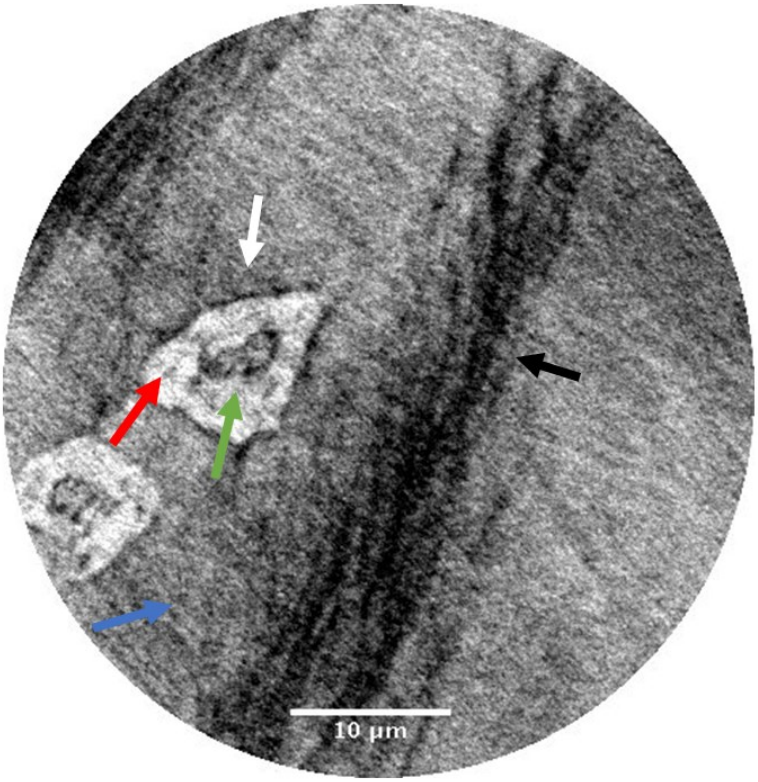

***Figure 3**:Close-up X-Ray image of OA cartilage: Chondrocytes (white arrow) with nucleus (green arrow) and shadow of cell organelles (red arrow) and fiber bundles of collagen with proteoglycans (black arrow) are visible. The blue arrow indicates the interterritorial martix*

In Figure 2 a non-degenerated (control, bottom) and a degenerated (top) samples are shown. Both 3D reconstructed sets come from the super-tangential zone of the cartilage. In A and B the fiber bundles are visible and are oriented in horizontal and vertical direction. The chondrocytes are sparsely distributed. Dark spots are visible in the lower part of the sagittal image.
In C and D the chondrocytes are visible but not a clear bundle structure. The chondrocytes are mostly oriented in the vertical direction (D). Dark spots appear but only around and inside the chondrocytes.

Figure 3 shows a close-up of a part of patella cartilage in the superficial-tangential zone. The different components of cartilage could be depicted on the acquired images: chondrocytes (white arrow) and fiber bundles (black arrow) are clearly visible.
Within the chondrocyte, the nucleus (green arrow) can be depicted and a shadow of cell organelles (blue arrow).
The blue arrow in figure 3 points out the interterritorial matrix.

Discussion:

In the non-degenerated samples (control) fiber bundles could not be clearly depicted. Though there are darker parts of the interterritorial matrix, they cannot clearly be identified as fiber structures as we are able to in the degenerated cartilage of the patella. The chondrocytes in Figure 3 do not show a circular structure but a triangular one, this might be a sign of degradation or resulting from cutting the sample (sign of deformation). The appearance of the dark spots is unclear, where they are coming from. We don't know if this comes from the samples themselves, e.g. age dependent calcifications or the preparation of those, e.g. some kind of precipitation.

Conclusion:

The data has a very high quality and shows structural details of the cartilage. More post processing with this data needs to be done to get meaningful medical information and knowledge. Investigations need to be done to understand the appearance of the dark spots and the deformation of chondrocytes. We like to continue this study at ID16A with a different protocol in sample preparation and handling.

Technical comments:

This second experiment was very successful. We need to optimize the sample preparation and treatment for future measurements. Technically-wise, the experiment did not encounter any major problems.

Acknowledgement

We are grateful to ESRF for the beamtime. We thank Yang Yang, the local contact, for all the work she did during the experiment, in the phase of data analysis and for the extra scanning parts of the sample in fluorescent mode. And we are thanking the whole ID16A team for extending our beamtime by half a shift.