Experimental report

28/03/2022

Proposal: 8-05-467 **Council:** 10/2020

Title: Exploring potential of neutron tomography for soft musculoskeletal tissues

Research area: Biology

This proposal is a new proposal

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Samples: soft tissue

Instrument	Requested days	Allocated days	From	To
NEXT	3	3	13/06/2021	16/06/2021

Abstract:

In this proposal, we aim to perform pilot imaging to evaluate if and how neutron tomography can increase our understanding of the structural response of soft musculoskeletal tissues during osteoarthritis. The tissues are articular cartilage, meniscus and ligaments from human knees with and without osteoarthritis. We will for the first time explore if local alterations in soft tissue microstructure and water content can be detected with neutron tomographic imaging.

Exploring potential of neutron tomography for soft musculoskeletal tissues
Experimental report – D50 NeXT – ILL, Grenoble (02/2020)

Experiment No.: 85332

A) Overview

Background: Soft tissue imaging is an unexplored application of neutron tomography (NT). New imaging options for soft musculoskeletal tissues such as meniscus and articular cartilage (AC) have potential application in furthering the understanding of joint diseases such as osteoarthritis (OA). NT has the potential to detect early signs of degeneration not visible on magnetic resonance images (MRI) or x-ray tomography as it directly visualizes the relative hydrogen content in the tissue.

Aims: Firstly, we aimed to explore what information can be obtained from NT images of AC and meniscus tissues, and how this imaging modality compares to traditional approaches such as MRI. Secondly, we aimed to compare the effects of different methods of AC and meniscus sample preparation on NT images.

***Method*:** NT imaging of human AC and meniscus samples of varying ages, prepared for imaging via four different protocols.

B) Experiment

Specimens: All specimens originated from the MENIX biobank, Lund University, Sweden. Meniscus specimens consisted of a wedge cut from the posterior horn of the meniscus. AC specimens consisted of rectangular plugs cut from larger plugs from the medial femoral condyle. Sample dimensions were around 5×5 mm, and varied from 4-8 mm in height. All specimens were stored at -80°C prior to the beamtime.

Preparation methods: The specimens were divided into four groups prepared according to the following:

- Fresh (AC, n=2) – Stored fresh frozen in phosphate-buffered saline (PBS).
- D_2O (AC, n=4; meniscus, n=2) – Soaked in D_2O-PBS for 2×4 h and 1×48h, changing solution each time.
- Fixed-wet (AC, n=2) – Fixed in formalin 48h, imaged in ethanol (99%).
- Fixed-dry (AC, n=4; meniscus n=2) – Fixed with formalin 7 days, dehydrated in ethanol (99%) and air-dried.

Imaging: The specimens were wrapped in Teflon tape and imaged two at a time in a custom Teflon sample holder according to parameters shown in table 1. The sample holder was sealed with a lid and constructed with a compartment for fluid (PBS, D_2O-PBS or ethanol depending on protocol) in the bottom to maintain humidity and hydration level without requiring immersion in liquid.

All specimens were imaged for using the “Regular scans” parameters detailed in table 1. Two AC specimens prepared in D_2O were selected for re-imaging with longer exposure and more averaging (“Long scan” in table 1) to explore possibilities of higher-quality images (figure 1).

In addition, two slices of one AC and one meniscus specimen were cut as thin as possible using a scalpel and radiographically imaged at higher resolution. The slices dried too quickly to sustain a long enough exposure time and did not provide useful images.

C) Ongoing analysis

Images are being analysed quantitatively to assess gradients in hydrogen density in the cartilage, an example is shown in figure 3. Comparative x-ray tomography and MR images of samples have been acquired and are currently being analysed to provide a comparison between samples of different ages and preparation methods.

D) Conclusions and feedback

During the beamtime, we preliminarily concluded that soaking in D_2O improved the image contrast within the cartilage, and that meniscus samples showed no internal contrast regardless of sample treatment. Fixed-wet samples could not be imaged in the wet state as the ethanol rapidly evaporated (see figure 2-C). It is unclear whether the lower-attenuation zone (seen in figure 2-C-D) within dried AC samples is the result of some property

of the tissue or a void forming as a result of drying. The longer imaging protocol improved image quality by reducing the level of noise.

The support during the beamtime by Dr Alessandro Tengattini was excellent, we received both valuable advice on which imaging parameters to use and on-site manufacturing of custom sample holders.

Table 1. Image acquisition parameters.

Neutron tomography – Regular scans			
FOV [mm]	14x14	**Exp. time [s]**	5
Total rotation [°]	360	**Nmb. Projections**	1216
Averaging	3	**Binning**	1
Pinhole [mm]	30	**Voxel size [µm]**	7.5
Imaging time [h]	5	**Reconstruction**	Absorption. RX Solutions X-Act Software
Neutron tomography – Long scan			
FOV [mm]	14x14	**Exp. time [s]**	10
Total rotation [°]	360	**Nmb. Projections**	1504
Averaging	5	**Binning**	1
Pinhole [mm]	23	**Voxel size/True res. [µm]**	7.5
Imaging time [h]	21	**Reconstruction**	Absorption. RX Solutions X-Act Software

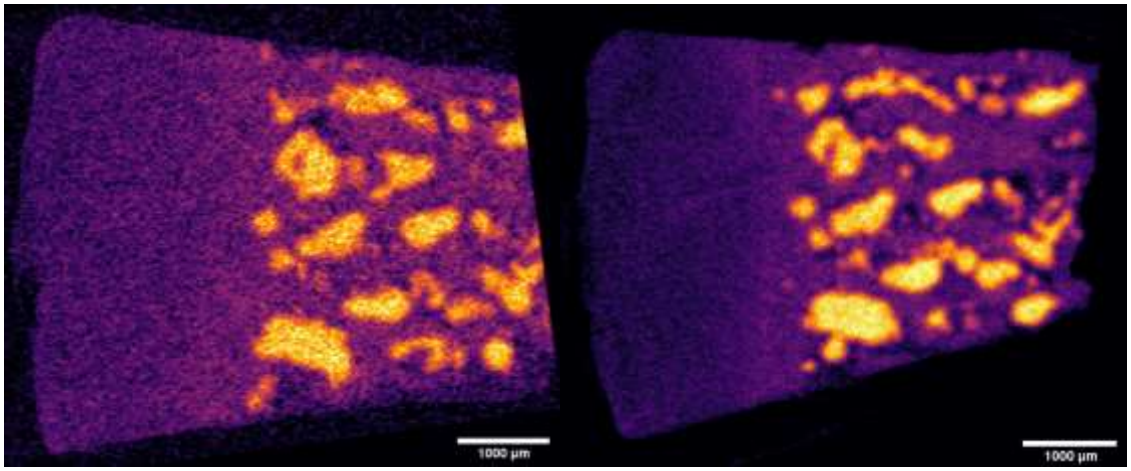

Figure 1. One AC sample that was imaged with both regular (left) and long scan (right).

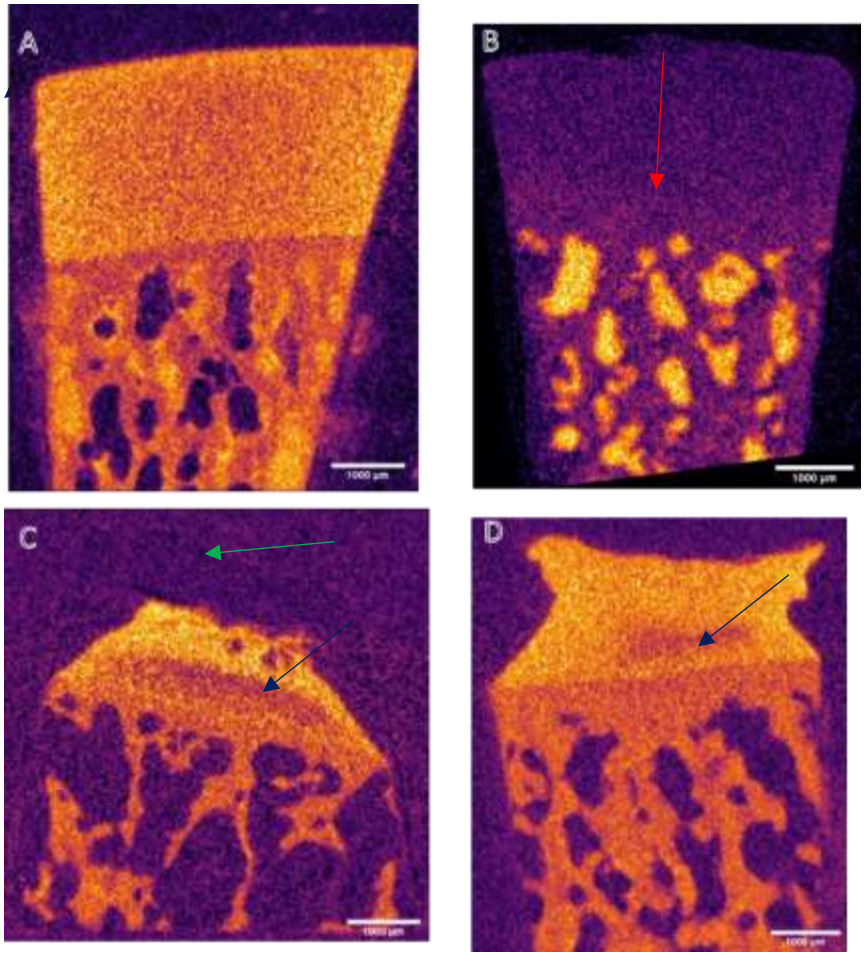

Figure 2. Comparison of the four protocols for sample preparations for AC: A – fresh, B – D_2O, C – fixed (wet), D – fixed (dry). The colors vary as in both the fresh and fixed samples the cartilage is the most attenuating tissue, whereas in the D_2O-treated sample the bone marrow is most attenuating. In B, a gradient of attenuation can be seen from the top to the bottom of the cartilage (along the red arrow). Note that C is reconstructed using only half of the acquired projections, as the drying of the sample produced too much movement for projections from the first half of rotation to be used. The outline of the pre-drying sample can be seen in the Teflon wrapping (green arrow). Both C and D exhibit a lower-attenuation central area characteristic of the dried samples (dark blue arrows).

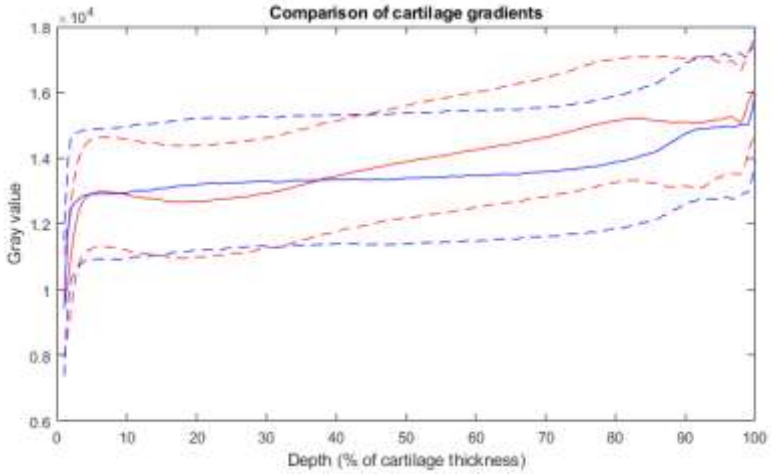

Figure 3: Gradient analysis of two AC specimens. Solid lines show the gray value averaged over the thickness of the sample, dashed lines show +- one standard deviation. Red is from an older donor (74 y), blue is from a younger donor (21 y).

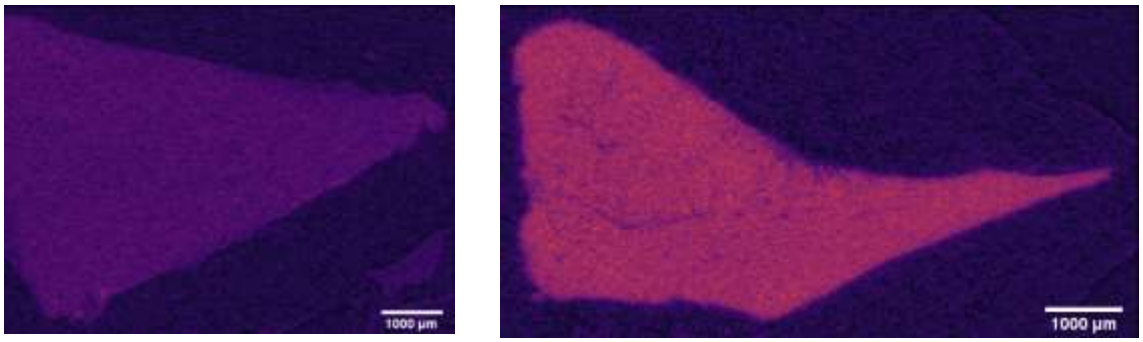

Figure 4: Imaging of meniscus samples treated with D_2O (left) and dried (right). No internal structures or gradients were visible except for cracks in the dried sample.