ESRF	**Experiment title:** Detection and characterization of microscopic diffuse liver pathologies in 3-dimensions with a macroscopic dark-field imaging technique	**Experiment number**: MD912
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The aim of experiment:
In the first part of the experiment (performed at ID19, see the other half of the report) we have utilized microtomography in order to select a series of liver sample at different stages of pathology. In this second part of the experiment, which represents the major part of the work, the ultra-small USAXS signal generated by selected samples was measured in tomography mode. The aim was to establish a relation between USAXS measured for large samples of the wavefront and properties of multiple microscopic structures inside the sample (extracellular matrix proteins, collagen fibres, etc.), which would normally won't be resolved due to low resolution of the detector system.

Experiment:
The alignment of the double crystal setup was performed without complications. As usual we have used 51 keV photon energy and Frelon detector system with 50×50 μm^2 pixel. However unlike in previous experiments, we started our tests without the MOCO feedback system. It was found that repeated rocking of the analyser crystal eliminates the typical systematic drift. The remaining instability was due to the X-ray beam – angular interval was adjusted every 10 minutes to compensate for it. It is important to notice that during this beamtime the stability of the main Laue monochromator was exceptional: we were able to estimate that the beam wavefront did not change its direction by more than 0.1 μrad in 20 minutes time interval.

A macro for the acquisition sequence was written and tested. Then we have continued tomographic imaging with the samples. In total 6 good CT data sets of selected liver samples were acquired together with many projection images of test phantoms and liver samples at 50×50 μm^2 and 100×100 μm^2 pixel sizes. The retrieval of 3 signals (attenuation, differential phase, and USAXS) per each pixel in every projection requires fitting of ~10^7 rocking curves.

Our algorithm, developed specifically for this purpose, processes such a data set in 15 minutes on single CPU with a necessary robustness (See Fig. 1).

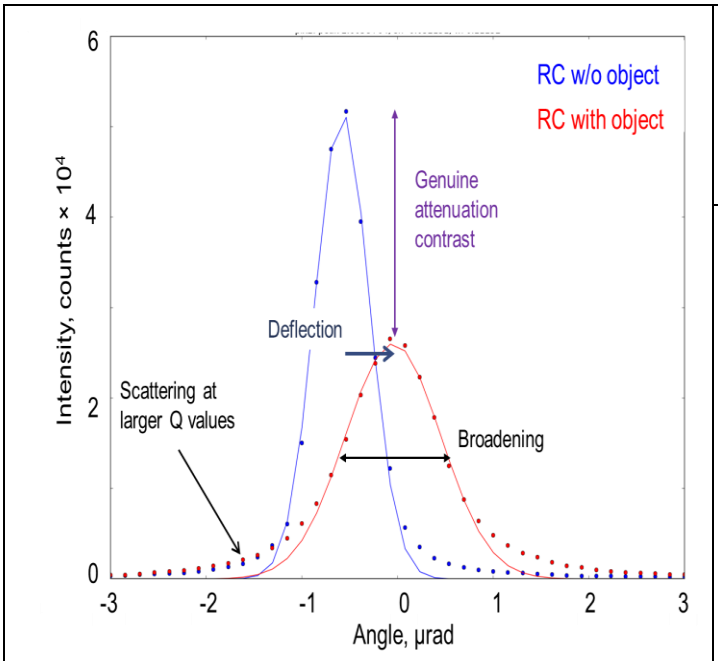

Fig.1 Crystal rocking curves for a 50×50 μm^2 wavefront sample: points – measured data, lines – computed model. One fit takes ~ 80 μsec.

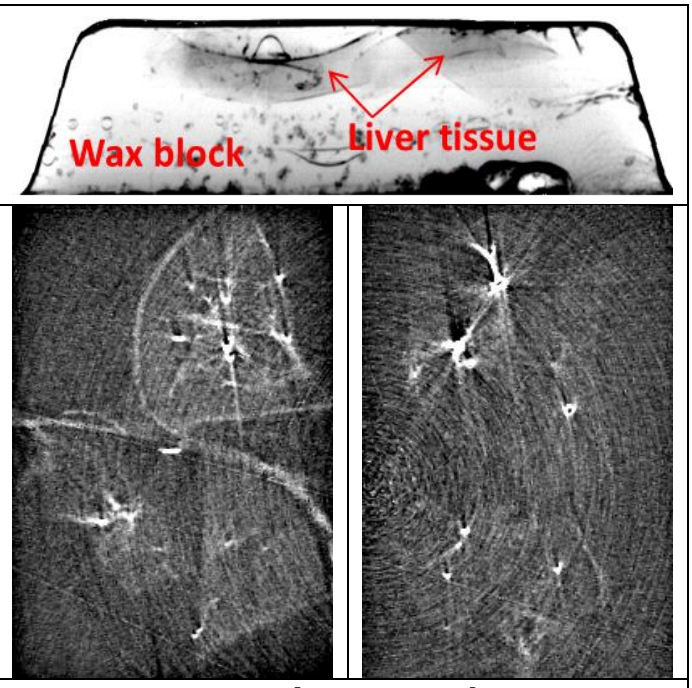

Fig.2. Top: extracted projection; bottom: one liver sample produces appreciable USAXS signal, while other (on the right) does not

Figure 2 illustrates an exemplary image, a projection, of a sample – liver tissue can be seen embedded in a large wax block. At the bottom panels of Fig. 2 a CT images of USAXS sources within the tissue is shown. Difference between two specimens is clearly visible albeit a strong scattering from the inner surface of blood vessels reduces image quality. Unfortunately we could have only used dried samples of rat livers from a histopathology data bank, while, in fact, the closer samples are to original "in-vivo" conditions, the better. A very high energy of X-rays used in the experiment must be exploited for imaging of a very large samples, for instance for examination of an entire pork liver.

Conclusion:

For the beginning we have managed to establish the USAXS-CT data acquisition protocol and successfully tested new reconstruction tools. Secondly, we have collected USAXS CT for four samples at different stage of fibrosis, for which μCT data are also available. It is unlikely that originally planned quantitative analysis of data is possible, since dried samples produces too strong parasitic scattering. However, the experiment was generally successful. It is shown that USAXS imaging with ~ 300×300 μm^2 spatial resolution can be used to detect deposition of extracellular matrix (a sign of developing fibrosis), which is otherwise only visible in a histopathological analysis or with an X-ray μCT method. Contrary to USAXS based imaging, the both latter cases the direct detection of fibrosis signs requires a spatial resolution of about of 2 μm, thus only very small specimens can be examined. We are also preparing a manuscript about the instrument and reconstruction tools, which should be of interest for researches from the scattering and the phase-contrast imaging communities.

Acknowledgement:

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