ESRF	**Experiment title:** Nano fluorescence/diffraction tomography maps osteocyte associated bone	**Experiment number**: LS-2654
Beamline: ID13	**Date of experiment**: from: 23.6.2017 to: 27.6.2017	**Date of report**:
Shifts: 12	**Local contact(s)**: Tilman Grünewald	*Received at ESRF:*

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Report:

Summary

We carried out a diffraction/fluorescence tomography experiment at ID13, aiming at characterizing the lacuno-canaliculiar network in bone (LCN) with a sub-100 nm spatial resolution. Thanks to the multi-layer laue lens setup (under development), we achieved a beam size of ~35 nm and a voxel resolution of 50 nm. We succesfully managed to collect a diffraction and fluoresence tomography dataset on a newly mineralizing sample, allowing us to the study the mineralization process around the LCN network by analyzing the mineral content, strain in the lattice and the presence of other mineral phases by diffraction tomography. XRF tomography will allow us to detect the amount of Calcium as well as the presence of other elements like Zn, which might play a hitherto undetected role in the bone metabolism. As this experiment was highly succesful, we wish to complete our sample set and measure data of two more samples on the progression of mineralization in a continuation of this experiment.

Samples and Setup

Setup: We were able to use the set of MLL lenses developed by Kubec, Niese, Keckes et al., which were just characterized previous to the experiment and proved to be highly performant. With this setup (see Figure 1), we managed to obtain a spotsize of 32 x 34 nm^2 at a flux of more then 1×10^{10} photons at 13 keV, which was an ideal beam for the purpose of this experiment. We needed however more time then anticipated to adopt the setup to the contraints of the very limited available space, which constrained us to measuring a single sample only. The beamline staff prepared new shielding and beam cleaning apertures as well as a new rotation stage setup based on a Smaract rotation stage. This was at the expense of 4 shifts of beamtime, however providing an uniquely

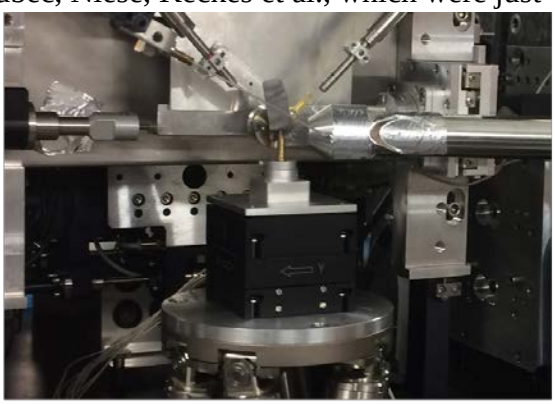

Figure 1 Tomography setup with the MLL setup

bright and small beam, therefore we consider it a very good investment of time. We furthermore settled all the stages to achieve a maximum of stability. From our preliminary data evaluation this was succesful and provided stability in the range of the beam size over the course of the experiment.
We used the Eiger4M detector to access a scattering vector of 5 to 35 nm^{-1} and a Vortex EM fluoresence detector for the XRF signal. As we could not use centering stages on the rotation axis (to increase the stability), we used an inverse centering algorithm developed by the beamline staff to center the sample by the piezo and the hexapod at each rotation angle.
Samples: From the sample set prepared, we were able to measure one sample with a very good resolution (slight angular oversampling to ensure reconstruction) in a complete dataset. This sample was a hypoparathyroidism affected bone from a patient treated by PTH, which re-initiated the dormant mineralization cycle. We prepared bone containing an osteocyte. The sample was about 3×3×3 μm^3 in size. The data collection of the sample took about 30 h.

Principal outcome

The diffraction sampled both wide angle diffraction and small angle scattering from the bone nanostructure. Fig 2a) shows the SAXS intensity of one projection. As the data was acquired without a defined center of rotation, the reconstruction had to be carried out in an interative way. To this end, we got support from the beamline staff, who used software developed by the PSI/cSAXS for a tomographic self-consistency alignment. We explicitely want to thank Manuel Guizar-Sicarios for this and are very grateful for the help. For our first reconstruction we used the total scattered intensity in the SAXS region for the alignment. The projections were normalized for beam intensity fluctuations and aligned. The sinogram of the reconstructed volume can be seen in Fig 2b) together with cuts through three planes as indicated in the figure. It is evident that we still have some residual uncertainty but will analyse the actual resolution in the near future.

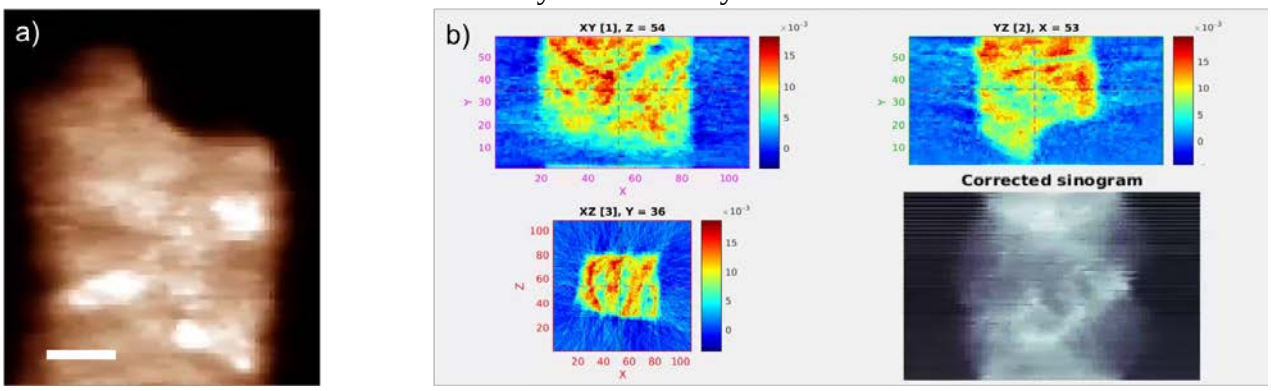

Figure 2. a) The mapped SAXS intensity of one projection showing the shape of the specimen. Scalebar is 1 µm and each pixel is 50 nm. B) Reconstructed volume based on total SAXS signal in three slices as well as the corresponding sinogram.

To put this achievement into context, the best published diffraction tomography has a resolution of a few µm, our own (unpublished) fluoresence record is 80 nm. We think that we managed to acquire a dataset of combined diffraction and fluorescence tomography which improved by more then one order of magnitude the existing published record.

We are able to identify the canalicular network from this dataset already in its current state and are highly optimistic that we can gain deep insights into the bone mineralization and remodelling process from this dataset and this method in general.

Conclusion and further proceedings

To conclude, we managed to collect a very good diffraction and fluoresence tomography dataset with unprecedented resolution of theoretically 50 nm voxel size. The true resolution is still to be determined. The success of this beamtime surpassed our own expectations and we managed to collect a dataset which is not only excellent in resolution but also shows scientifically extremely interesting phenomena such as the mineralization around the canaliculi network. The data is currently in the process of being evaluated but from the current status we anticipate rapid publication in an appropriate journal.
As we however managed to measure only one sample (which took about 30 h), due to the long setup time and beam dumps, we would like to complete the dataset with two additional samples and will apply for a continuation of the experiment.