ESRF	**Experiment title:** Imaging bone's 3D nanostructure in brittle bone disease	**Experiment number**: MA-2833
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Report:

Human bone generates mechanical integrity through its multi-length-scale structure. Specifically, strength develops at small length-scales through sliding mechanisms within and between fibrils [1]. These fibrils are a composite of nano-sized collagen molecules and mineral platelets, with larger aggregates of mineral deposited on the surface of the fibrils, termed extrafibrillar mineral. Many bone diseases result in an increased risk of fracture, precisely due to changes in the multi-length-scale structure that affect the material's mechanical resistance [1].

As the fibril length-scale is critical to generating strength, there is a need to image bone at the nano-level to understand how disease-related changes in collagen and mineral result in reduced plasticity and increased fracture risk. Here, we have used coherent diffraction x-ray imaging (CDI) at the ESRF, which reconstructs the 3D structure of micron-sized samples at a voxel size of 15-33 nm.

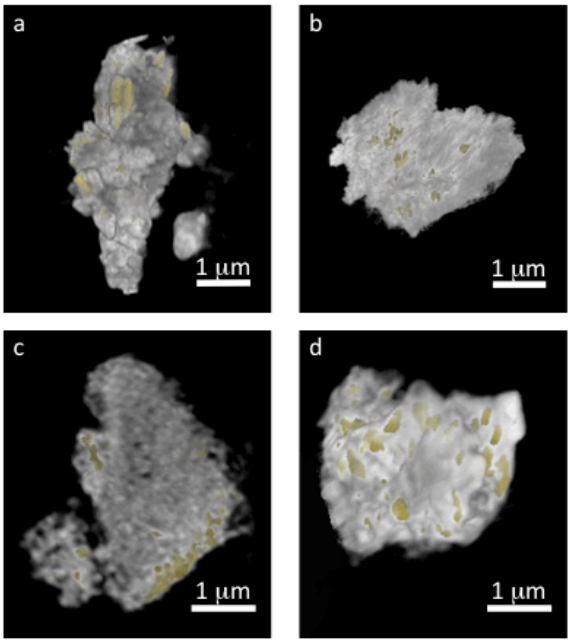

Figure 1: 3D CDI reconstructions of the nano bone structure, where the bone matrix is grey and the large extrafibrillar mineral is yellow. a) Here, the 14-year-old healthy cases have large mineral platelets, b) while the 18-year-old OI type I case has smaller mineral platelets. c) The healthy 2-month-old bone has a lower density of mineral crystals than in the d) fetal bone from a type II OI case.

In our experiments at the ESRF in September 2015, we used CDI to investigate the 3D structure of human cortical bone from cases of osteogenesis imperfecta (OI), which is also known as brittle bone disease. OI is caused by mutations in the genes that synthesize collagen and affects the collagen structure as well as the mineralization of bone [2]. OI has a range of severities based on the mutation, *e.g.,* type I is the least severe with an elevated number of fractures and type II is lethal at birth with numerous fracture *in utero*. Here, we investigated human femoral cortical bone from fetal cases with OI type II (n = 2) and an 18-year-old case with OI type I (n = 1). Our previous CDI results from the ESRF (September 2014) on healthy femoral cortical bone from 2-month-old and 14-year-old cases were used as controls.

Femoral cortical bone samples of roughly 5 μm^3 were cut with a laser microdissector at the ESRF and deposited on Si_3N_4 membranes. The samples were measured via tomographic scans at the ESRF beamline

ID10 with 8 keV coherent x-rays. A phase retrieval algorithm was applied to reconstruct the 3D electron density distribution from the 3D Fourier intensity data with a 15-33 nm voxel size.

From the reconstructed 3D images of the bone fragments, we were able to observe differences between the healthy (Fig. 1a) and type I OI (Fig. 1b) cases. Here, in the healthy bone tissue, large extrafibrillar mineral crystals are visible outside of the fibrils. However, while the overall mineral density in the type I OI cases was similar to the healthy bone, no large extrafibrillar mineral crystals were observed (Fig. 1b). Type I OI clinically leads to increased bone fracture risk. While the exact role of the extrafibrillar mineral is unknown, it may play a role in generating plasticity through shearing of the matrix. The lower collagen content and reduced size of the extrafibrillar mineral crystals could be the source of increased fracture risk in OI type I [3,4].

For the most severe type II OI cases, the 3D images from healthy (Fig. 1c) and OI (Fig. 1d) cases show that the bone is more mineralized in OI. This result is supported with data from the literature showing that mineralization increases with OI severity [3]. Looking closer at the 2D bone matrix, the spacing beween the collagen fibrils is greater in the OI type II cases (Fig. 2), which indicates that something could be wrong with the 3D structure of the fibrils.

From our results at the ESRF at the ID 10, we are in the final stages of manuscript preparation from our results on healthy bone acquired in 2014. Additionally, we are analyzing the data and preparing a manuscript on the effects of OI from our ESRF experiments in 2015. Our CDI experiments at the ESRF supplement histology, fourier transform infrared imaging, and mechanical testing to characterize the bone structure, composition and mechanical properties at multiple length-scales. From these experiments, we aim to understand the role of extrafibrillar mineralization in bone's mechanical integrity as well as the disease-related changes to the nanolevel structure associated with OI.

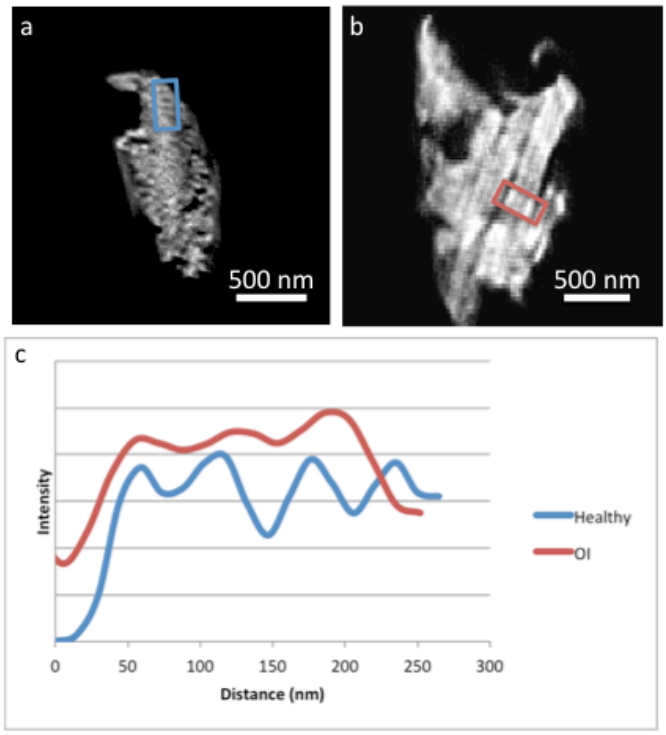

Figure 2: 2D slices from the CDI reconstructions of the bone nanostructure are shown for the a) 2-month old healthly case and the b) fetal OI type II case. In both of these images, collagen fibrils are visible. By performing a line scan across the fibrils in the blue boxes, the distance between the fibrils can be measured by c) plotting the intensity as a function of distance, where the peaks represent the position of the fibril. Here, the OI disease cases are found to have a larger distance in between fibrils.

References

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