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| ESRF | **Experiment title:**<br>Bone cells-matrix interaction: nano-SAXS/XRD analysis of the perilacunar mineral structure | **Experiment number**:<br>SC-4105 |
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## Report:

We performed scanning X-ray small-angle scattering experiments with a nano-focused beam on 200 nm thin sections of bovine bone. The aim of the experiment was to reveal difference in the local structure of the bone matrix surrounding osteocytes and to characterization which structural properties are altered locally.

The bone sections were prepared beforehand by ultramicrotomy cutting using a diamond knife. The bone sections were carefully placed on silicon nitride membrane windows, which served as substrate for X-ray experiments. At the ID13 beamline the samples were glued to brass pins and mounted on the sample stage allowing the samples to be scanned with high positional precision in the X-ray beam.

X-ray measurements were performed in transmission geometry at a photon energy of 14.85 keV. The beam was focused to about 200×200 nm² (horizontal×vertical) using crossed nano-focusing refractive lenses, yielding a primary beam intensity of about $1.1\times10^{10}$ photons/s. The samples were aligned in the focus of the beam using the visible-light microscope (compare Figure 1a) and two-dimensional mesh scans were performed on different areas of the samples with typical exposure times of 1 s per scan point and 200 nm step size. The need to scan large areas in combination with a long exposure time per scan point led to the total

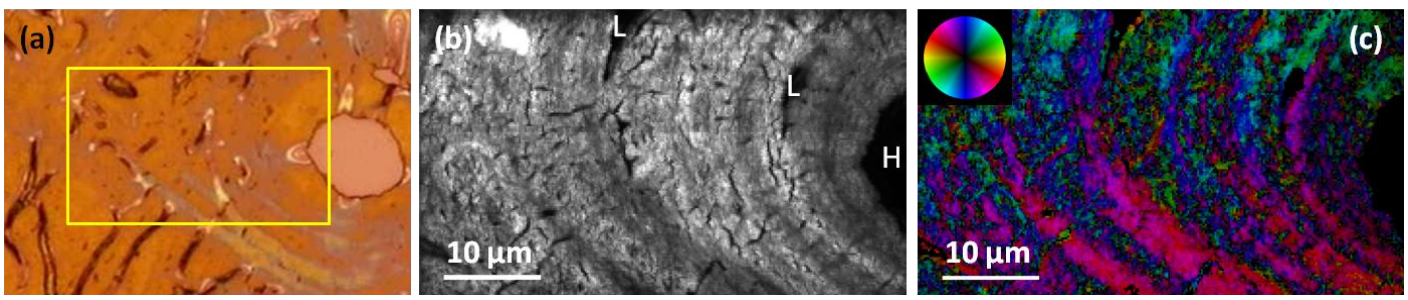


**Figure 1: (a)** Microscopy image of the 200 nm bone section taken with the beamline microscope before the measurement. The scan area is indicated by a yellow box. **(b)** Map of the total SAXS intensity for a scan with 200 nm step size. H: haversian canal, L: lacunae. **(c)** Orientation (color) and degree of orientation (intensity) of the mineral platelets in the bone section.

scanning time of several hours per mesh scan. The scattering signal was recorded on the Eiger 4M detector (75×75 µm² pixel size, 2167×2070 pixels) positioned at the sample-to-detector distance of 0.947 m. The primary beam was blocked by a beam stop and a small flight tube filled with Helium was inserted into the beam path between the sample and the beam stop to avoid scattering from the air.
Real space images of the scanned areas were obtained during the beamtime by integrating the total scattered intensity in each scan point as shown in Figure 1b. Here, variations of the gray level correspond to different scattering strengths in the sample. Canaliculi with diameters on the order of the beam size can be identified as thin dark lines pointing radially outwards from the haversian canal (H). The local orientation and the degree of orientation of the mineral platelets was determined from the individual scattering patterns using a python routine based on pyFAI [1] for azimuthal regrouping of the scattering data. A typical scattering pattern is presented in Figure 2a and a map of the orientation and the degree of orientation is shown in Figure 1c. Sub-µm variations of the orientation can be identified.

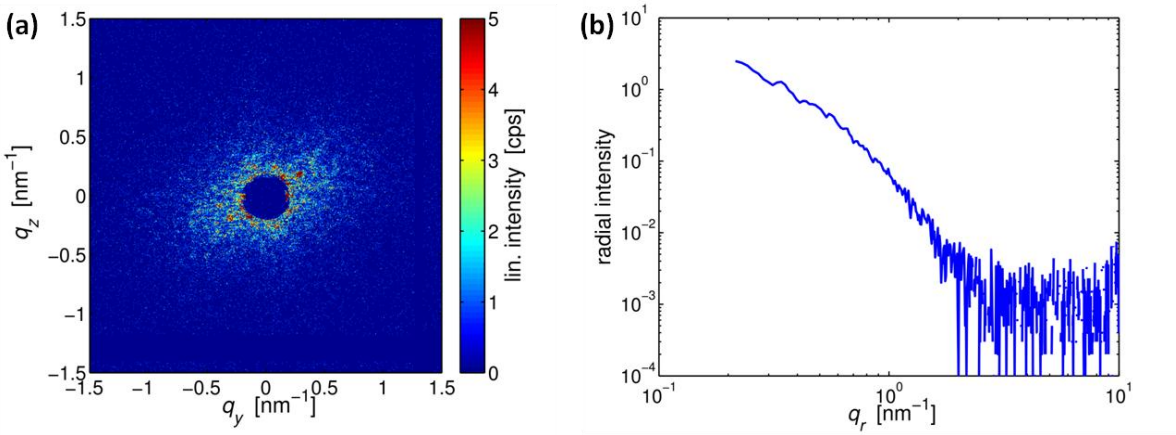


**Figure 2: (a)** Typical example for a scattering pattern and **(b)** the corresponding azimuthally integrated intensity profile obtained from a 200 nm bone section with 1 s exposure time.

In the next step of the data analysis the so-called T parameter, which gives an estimate of the smallest dimension of the bone platelets, was calcualted from the azimuthally integrated data using a python based routine [2]. Due to the (partial) coherence of the X-ray nano-beam, the radial intensity profile is not completely smooth, but shows variations related to the speckles in the scattering pattern (see Figure 2b). However, to compare the nano-SAXS experiments to conventional (micro-beam) SAXS experiments, the same routines for analysis were used. Figure 3a shows a map and Figure 3b a histogram of the obtained T parameter. The distribution of T parameter has a maximum at about 4.2 nm which compares well to conventional SAXS experiments where T parameters on the order of 2-5 µm are observed.

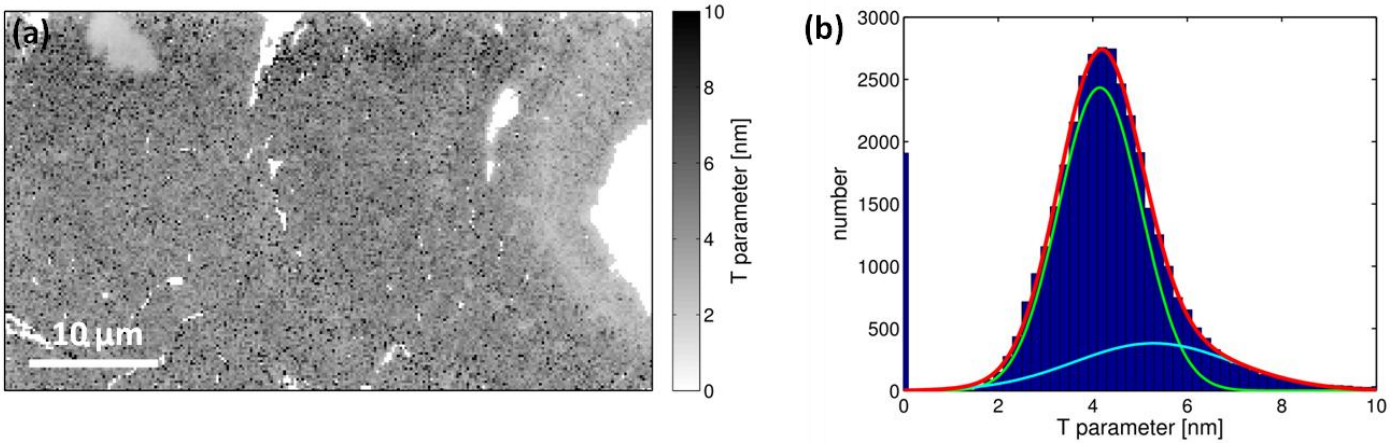


**Figure 3: (a)** T parameter calculated for the scan shown in Figure 1. **(b)** Histogram of the distribution of T parameters in panel (a) along with the fit of a superpostion of two gaussians to the histogram (red: superposition; green, cyan: individual gaussians).

[1] Kieffer & Karkoulis, J. Phys. Conf. Ser. (2013);
[2] Gourrier, unpublished