ESRF	**Experiment title:** High resolution X-ray tensor tomography to study the spiral nanostructure of narwhal tusk	**Experiment number**: SC-5257
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Report:

The aim of the experiment was to analyse the orientation and spatial distribution of the collagen and mineral platelets in narwhal tusk with micro resolution, with the final goal of connecting the nanostructure of such tissue with the macroscopic helicoidal structure. We hypothesize that the orientational arrangement of collagen fibrils and biomineral nanocrystals is directly related to the spiral nature of the tusk and its mechanical properties. Thereby its determination is an essential piece of the spiral puzzle of narwhal tusks. Previous experiment revealed an important relationship between the orientation of the nanostructure at different positions in the tusk and the macroscopic spiral shape; however, a microbeam is necessary to achieve higher resolution and fully resolve the nanostructure in the three-dimensional space.

High-resolution small- and wide-angle X-ray scattering tensor tomography (S/WASTT) was done at the microfocus beamline (ID13) of the European Synchrotron Radiation Facility (ESRF, France). An X-ray beam of 13.0 keV was produced using a channel-cut Si(111) monochromator and focused to 3×3 μm^2 using a pair of Pt-coated fixed elliptical Kirkpatrick–Baez mirrors (KB) and a set of beryllium compound refractive lenses. A small flight tube filled with He was placed between the sample and the detector to minimise air absorption and scattering after which there was a small beamstop to block the direct beam. The scattering signal was recorded using an Eiger X 4M detector (Dectris AG, Switzerland) placed downstream from the flight tube. The 3D samples were prepared on PMMA pins to minimise X-ray absorption and in a dual-axis goniometer that allows for rotation (α) around a tomography axis, tilt perpendicular to the tomographic axis (β), and scanning in the x-y plane [1]. The 3D samples were spatially mapped with a step size of 3 μm and an exposure time of 5 ms. Approximately 400 projections were measured for each sample at 6 tilt angles (β) between 0 and 40 degrees and rotation angles (α) between 0 and 180 degrees for a tilt of β=0° and 0 to 360 degrees for β≠0°. The measured 2D projections were aligned using an iterative alignment method [2] and the reconstruction of the reciprocal-space map was carried out following a procedure reported in Nielsen et al., [3] based on that described by Liebi et al. [1, 4]. The robustness of the reconstruction was checked by visual comparison of 2D orientation,

anisotropy, and degree of orientation between the measurements and simulated projections of the reconstructed data. The degree of orientation was calculated as the ratio between the isotropic tensor coefficient, and the full tensor [1].

The reciprocal space map of the scattering signal corresponding to the mineral particles (q = 0.36 – 2.25 nm^{-1}) was reconstructed using SAXS tensor tomography with a cubic voxel size of 3 µm. The sample was obtained from a segment in the upper half of the tusk (14 – 28 cm from the tusk tip) containing cementum and dentine. Previous experiments indicated a reciprocal space map with multiple orientations per voxel. For that reason, higher resolution SASTT was done reducing the voxel size from 25 µm to 3 µm (Figure 1). Two differentiated regions possessing low and high degree of orientation, cementum and dentine respectively, are clearly visible in Figure 1a. The orientation of the mineralised collagen fibres, represented by the direction of the streamlines, is mostly aligned in the longitudinal direction of the tusk. The orientation of the reconstructed reciprocal space map shows the consistent axial orientation in the dentine (Figure 1b). In the cementum region (Figure 1c), an overall lower degree of orientation is found, but some areas with more complex orientation are visible. Some variations from the axial direction can be observed in the outer surface, where vertically oriented domains appear embedded in an axially oriented matrix.

A manuscript is under preparation, containing the results of the experiment as well as previous results using 2D scattering and birefringence microscopy.

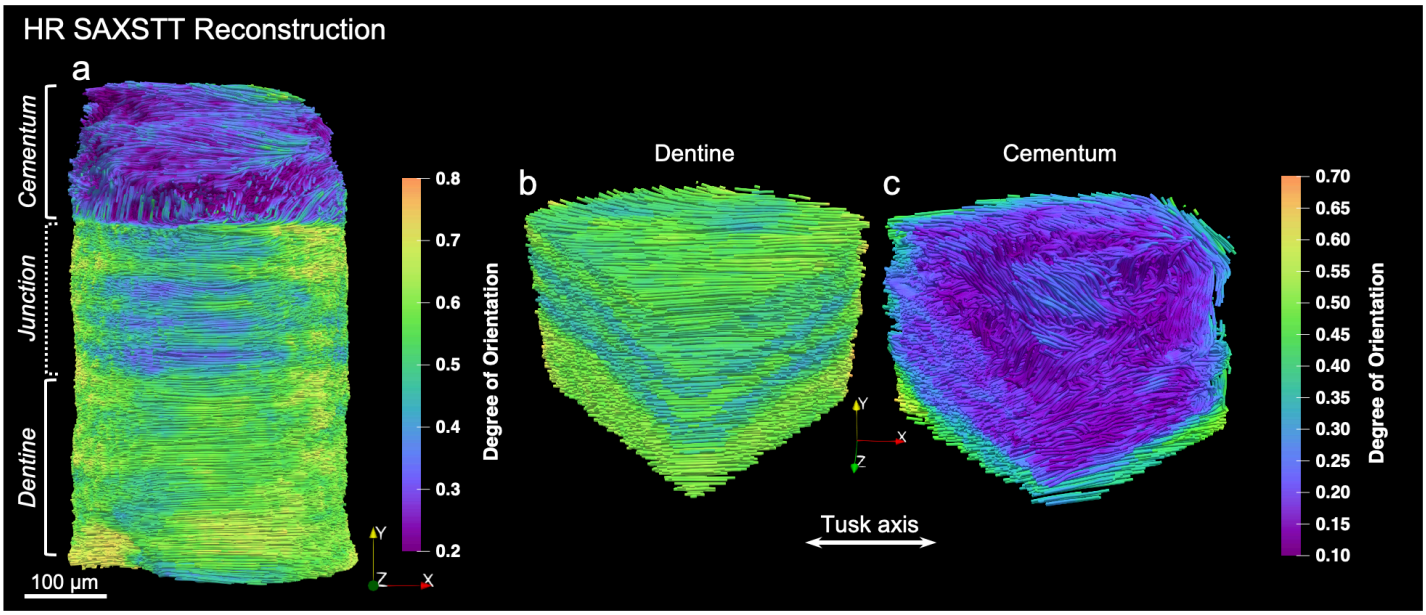

Figure 1. 3D representation of the mineralised collagen fibrils' orientation in the narwhal tusk. The reciprocal space map of the scattering in a q-range corresponding to the mineral particles was reconstructed using SAXS tensor tomography with a cubic voxel size of 3 µm. High-resolution SAXS tensor tomography of narwhal tusk (a) and two subvolumes in the dentine (b) and cementum (c) regions.

References

[1] M. Liebi, et al., *Acta Crystallographica A*, **2018**, 74 (1), 12-24.
[2] M. Odstrčil et al., *Opt. Express*, **2019**, 27 (25), 36637-36652.
[3] L. C. Nielsen et al., *Licentiate thesis, Chalmers University of Technology*, **2022**.
[4] M. Liebi et al., *Nature*, **2015**, 527 (7578), 349-352.