ESRF	**Experiment title:** Mapping the forming narwhal tusk by large volume multiscale X-ray imaging	**Experiment number**: Ls - 3114
Beamline: BM05	**Date of experiment**: from: 3/11/2022 to: 6/11/2022	**Date of report**: 16/02/2023
Shifts: 12	**Local contact(s)**: Philip Cook	*Received at ESRF:*

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Report:

The aim of the experiment was to visualize the growth of the narwhal tusk structure inside the growth chamber in the skull with a hierarchical computed tomography imaging scheme. This approach would allow for scanning the entire skull with a lower resolution, and from this overview select regions to scan at higher resolution. This should make it possible to understand the structures on multiple biologically relevant length scales. The experiment bears a major role in fully determining the growth of the erupted narwhal tusk, and how the growth is controlled in the growth chamber in the skull of the narwhal. Of special interest in this regard is for the formation of the homochiral spiral tusk structure. With the tremendous size of the sample, it is only possible to obtain all relevant information and biological interplay using such an approach.

Hierarchical phase contrast computed tomography (HiP-CT) was used to analyse two skulls of male narwhals, each containing an erupted and an embedded tusk at the BM05 beamline at the European Synchrotron Radiation Facility (ESRF, France). In total four scans at a resolution of 25 micrometres were completed, providing an overview of the two skulls. Ten regions of thin tusk, skull and thicker tusk were selected from these and scanned at a voxel size of 6 µm. Furthermore, four regions, at the beginning of tusk growth and at a point where the tusk was thicker, were scanned at 1.6 µm voxel size. CT scans with 25 and 6 µm voxel sizes were achieved with an X-ray beam with an energy of 106 keV. CT scans with 1.6 µm voxel sizes were achieved with an energy of 91 keV. In all cases a half-acquisition scanning mode was used to vastly increase the field of view. It was possible to cut down on scan time by effectively using the sheer size and thickness of the sample as a beam attenuator, thereby allowing for use of higher flux and as an effect greatly lowered exposure time. Reconstructions were run as part of the pipeline at the beamtime enabling the selection of regions of interest based on overview scans. Three examples of these scans are shown in figure 1, one at each voxel size employed. The figure shows the remarkable possibilities at BM05 of scanning a full volume at a voxel size of 25µm, and afterwards use this for making scans at higher resolution.

The final reconstructions unfortunately show some ring artefacts and varying intensities throughout a stack. These artefacts become very apparent when stitching the reconstructed stacks, showing as bright bands. We are currently working on ring removal on the finished reconstructions and intensity normalization will be explored to normalize the data set. In spite of these challenges, the data are clearly very promising and reveal new detailed information on the tusk formation site in the narwhal skull.

The data have for example already confirmed that tusk formation starts at the interface between cementum and dentine. We expect that the data will help formulate a model tusk formation and the formation of the enigmatic spiral structure of the narwhal tusk.

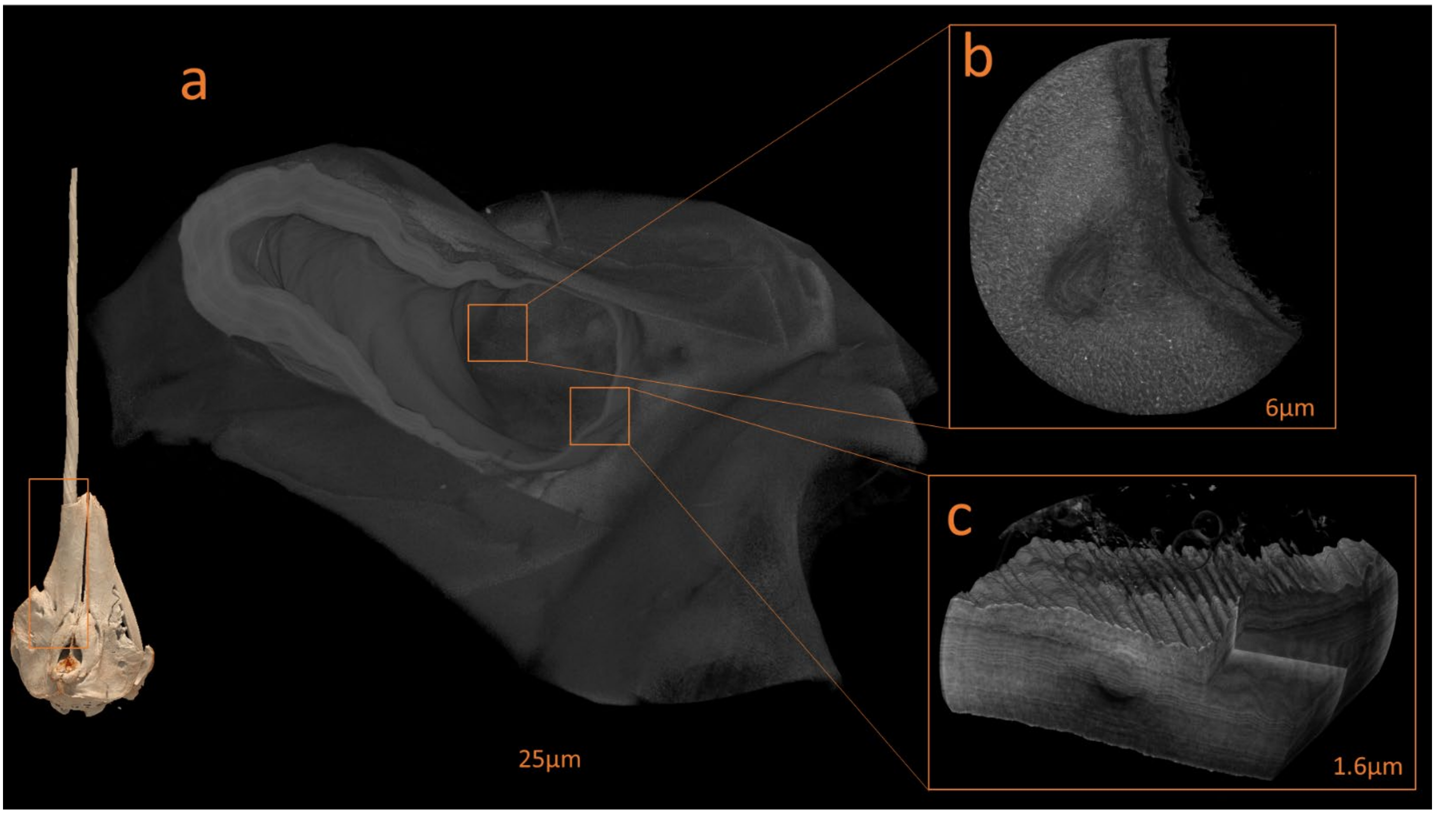

Figure 1. 3D view of three different computed tomograms measured during the beamtime. Insert to the left is a CT reconstruction from a clinical measurement of the same narwhal measured at BM05 before cutting. a is the full volume measured at 25 µm, where the two volumes to the left b and c are zoom ins at regions of interest at 6 and 1.6 µm respectively.