ESRF	**Experiment title:** *Nanometer length-scale distribution and arrangement of mineralized structures within human tooth dentine: An ultra high-resolution holotomographic and ptychographic study*	**Experiment number**: MD720
Beamline: ID22ni	**Date of experiment**: from: 30 Jan 2013 to: 05 Feb 2013	**Date of report**: 24.8.2013
Shifts: 18	**Local contact(s)**: Peter Cloetens	*Received at ESRF:*

Names and affiliations of applicants (* experimentalists):

***Paul Zaslansky, * Jean-Baptiste Forien**:
- Julius Wolff Institut, Charité - Universitätsmedizin Berlin, DE

***Peter Cloetens:** ESRF 6 rue Jules Horowitz B.P 220 F - 38043 Grenoble Cedex, FR

***Franz Pfeiffer, *Irene Zanette, Marco Stockmar, Pierre Thibault:**
- T U München Physics-Department E17 D - 85748 Garching, DE

Report:

We study the adaptation of human teeth to their mechanical, load-bearing function. The mineral in teeth is the main structural component contributing to tooth stiffness and it is found in 2 main forms in tooth dentine: an intertubular bone-like matrix and surrounding many of the cell-extension tubules of teeth (spaced about 10 micrometers apart), in highly-mineralized peritubular sheaths. To this end, tooth dentine is a biological nano-ceramic composite that forms a tubular micro-architecture with 3 very different compositions: empty voids (in tubules), highly mineralized tubules (4~5 micrometer in diameter, 1~2 micrometer thick rim) and bone-like matrix [1]. In a typical sub-volume of dentine tissue, all 3 components are found within volumes as small as 10 cubic micrometers. We proposed to image representative volumes in dentine so as to better understand the 3D arrangement of the juxtaposed mineralized tissues, and to use cutting-edge 3D nanotomography and ptychography to better understand the mineral distribution and arrangement.

During the MD720 experiments on ID22NI (Feb 2013), needle-like samples of human dentine of known orientation were imaged (Fig 1). The samples were taken from sound teeth (extracted for clinically justified purposes, unrelated to this experiment). Voxel resolutions in the range of 25~400 nm and the 17 keV KB mirror arrangement were used (pink beam; 1.5% monochromaticity). Two types of experiments were performed:

a) X-ray magnified phase tomography (nano-holotomography, HT) using multiple (3 or 4) propagation distances, as detailed for example in [2]. These were performed at low and high magnifications, attempting to couple between observing the overall context of structures in relation to the sample geometry (given in low resolution) and region-of-interest tomography using very high magnifications (beneath 50 nm effective pixel size).

b) measurements of the same samples using the near-field ptychography approach, currently under development by the TUM group in Garching, Germany.

During the experiment, we collected repeated measurement data from crown and pulp regions of molar and premolar human teeth (in the final data we have more than 150 complete tomographic scans). Intensive data processing on both the ESRF OAR cluster and the dedicated TUM (portable) cluster was needed to refine the type and quantity of data that we could obtain during the experiment. All in all, more than 3 TB of data was collected with repeated measurements used to assess reproducibility.

While the HT phase-retrieval code e of ID22 [3] is the main available tool for retrieving phase radiographs (after extensive testing, we converged to using a delta/beta ratio of 202 for dentine), the ptychographic phase-retrieval is being performed by the TU-Munich team and will require substantial processing time. Both retrieval processes are very computational expensive, the latter also requiring further algorithm refinement.

A main observation for the holotomo phase-retrieved data has shown that low resolution (non-local) reconstructions are prone to strong phase-retrieval artifacts mainly near sample edges due to the large difference in density between the samples and the surrounding air (see example cross-section slice shown in Fig 2). Consequently, strong 'ringing' effects arise, rendering the quantitative quality of the low resolution data (in terms of true electron density) questionable. Surprisingly however, and although lacking a baseline (DC) phase shift component, the local tomography images of dentine at high magnification (50 nm and below) show exquisite details (Fig 3). In some datasets - when phase retrieval is successful due to minimal radiation damage and negligible sample motion - artifacts are minimal. Here, the arrangement of the peritubular dentine cuffs, the intertubular matrix and even traces of collagen fibers are observed. The retrieved density and degree of mineralization require validation and will be further evaluated when ptychographically-retrieved mineral densities become available after extensive (year-long) data processing. This will not only help us better understand the micro architecture of teeth, but also help better understand how phase-retrieval can be improved or tuned to better quantify mineral density in bone-like tissues.

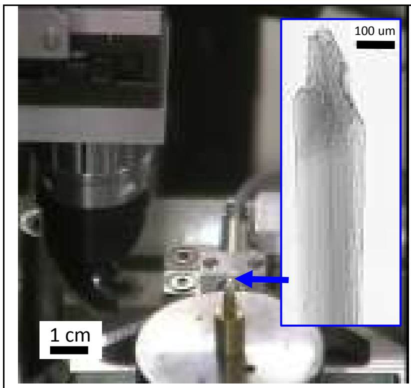

Fig 1: Typical sample, mounted along a crown-pulp axis on the rotation stage positioner of ID22ni

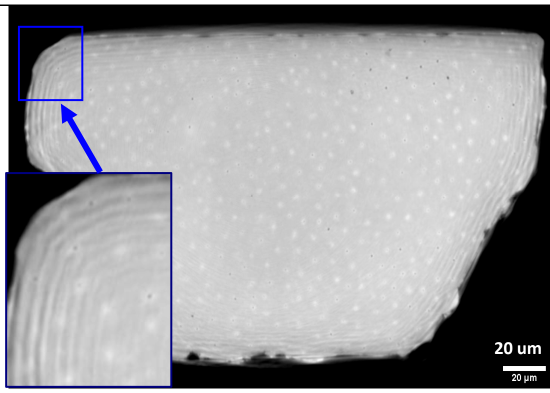

Fig 2: Reconstructed HT-phase-retrieved cross-section reveals the distribution of dentine tubules accompanied by retrieval 'ringing' near edges

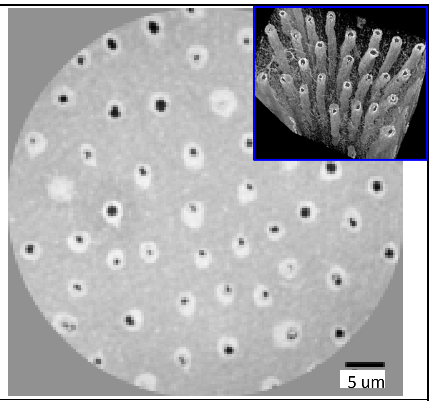

Fig 3: In local (region of interest) tomography, exquisite 2D structural detail is seen (rendered 3D in inset)

[1] Zaslansky P., 'Dentin' in *Collagen: Structure and Mechanics* (ed P. Fratzl), 421-442 Spinger (2008).
[2] Langer M, Pacureanu A, Suhonen H, Grimal Q, Cloetens P, et al. (2012) X-Ray Phase Nanotomography Resolves the 3D Human Bone Ultrastructure. PLoS ONE 7(8): e35691.
[3] Cloetens P, Ludwig W, Baruchel J, Van Dyck D, Van Landuyt J, et al. (1999) Holotomography: Quantitative phase tomography with micrometer resolution using hard synchrotron radiation X-rays. Appl Phys Lett 75: 2912–2914