| ESRF | **Experiment title:**<br>Why teeth hit hard: Understanding the structure-function relationship in teeth by white-beam energy dispersive 3D texture determination | **Experiment number**:<br>SC 4249 |
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## Report:

## Summary


We successfully applied our very recently developed technique of 3D texture scanning by energy dispersive Laue diffraction (EDLD) for the elucidation of crystallographic texture in ancient teeth (mature and developing). We used a white x-ray beam and energy dispersive 2D detector to gain direct 3D texture information without rotation. Taking advantage of our proof of prinicple experience from our very first EDLD texture experiment last year, we were now able to map significant areas of enamel and dentine as well as the enamel dentine junction (EDJ) with a position resolution of 40 μm. We obtained 3D diffraction patterns clearly showing the variation of the 002 reflection orientation as well as areas with two combined directions of preferred orientation. As a major advantage of EDLD texture measurements over monochromatic diffraction, also out of plane variations of orientation could be directly observed. For mapping with even higher spatial resolution below 40 μm, a focused beam would be required.


## Samples and Setup

The aim of this experiment was to apply our recently developed EDLD texture measurement technique to teeth as highly mineralized, but very complex biological tissues with highly oriented crystallites consisting of hydroxy apatite (HAP, calcium phosphate). This was an important step following our proof of principle experiment employing a carbon fiber phantom sample last year towards an application of true scientific interest. We were successful in obtaining 3D texture results from 3 tooth samples from archeological origin: 2 mature teeth and 1 developing tooth, all ground to a thickness of approx. 10 μm and exhibiting the tooth cusp with the highly mineralized enamel as the outside layer and less mineralized and softer dentine underneath, see Fig. 1.

The setup used a white beam comprising the full white bending magnet spectrum, attenuated only by the Be windows in the beam line, giving a lower energy cut-off at about 4 keV. The beam size was reduced down to 40 µm by 3 sets of beam defining slits. A smaller beam would have resulted in too low flux. The only solution would have been a focusing optics, which was not available. Therefore the beam was too large to also scan sub-surface lesions in addition to healthy teeth, as originally planned, because they are only about 50 µm in depth. We therefore concentrated on the texture of mature and developing teeth and the EDJ, which is nicely resolved at this spatial resolution.
The energy dispersive 2D detector (SLcam) was mounted on the diffractometer arm, so it could be rotated around the sample in order to cover a wider range of scattering angles. Due to an improved geometry involving a further reduced sample-detector position in the already very tight setup the measurements could be carried out with 2x2 detector positions as compared to 4x4 positions in the first experiment, which resulted in a great gain of speed. Together with the reduced exposure time of 60 s due to the strongly scattering sample, several texture maps of 800x800 µm and a number of line scans across the EDJ could be carried out.

**Figure 1: Tooth cusp of a mature tooth. Bright region: enamel. Dark region: dentine. Scale bar: 0.5 mm**

## Principal outcome

Variation of the direction of preferred orientation of the tooth mineral (HAP) crystallites could be imaged as a function of position in the tooth. Figure 2 shows a linescan with 60 µm resolution and the progressively changing texture of the HAP 002 reflection. It can be immediately seen that not only the azimuthal position and the width changes with position, but also a splitting into two main directions (image 3 from letft) occurs along with a shift of intensities between upper and lower ring which indicates tilt out of the detector plane. Any effects out of the detector plane cannot be directly observed with conventional monochromatic diffraction, which demonstrates the advantage of EDLD texture scanning.

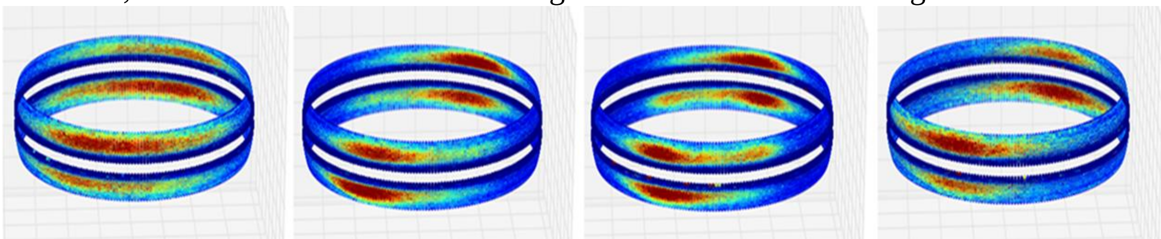

**Figure 1:The tilt and co-orientation of two crystal axes in developing enamel. A linescan with 60µm resolution shows the subsequent tilting and dealignment of two crystal axes in teeth as measured by the orientation of the 002 reflection of hydroxyapatite.**

Our preliminary data analyisis showed several regions of combined preferred orientations of the 002 reflection along at least two distinct axes. We are currently in the process of evaluating and fitting the data further and expect very useful information about the formation of teeth.

## Conclusions and further proceedings

We successfully showed that our very recently established technique of EDLD texture scanning with a white beam and energy dispersive detector can be applied for texture mapping of complex biomineralized tissue. Due to the direct 3D information within the available window of energies and diffraction angles we could directly observe rotations and tilts of the 002 reflection also out of the detector plane and could directly image combined orientations.  Since we were already able to greatly improve the setup and thus saved considerable amounts of measurement time as compared to the first experiment, we are very confident about the further application of this method to other complex mineralized materials. Specifically teeth proved to be a very promising system as they exhibit very interesting texture patterns and yield a strong diffraction signal at very reasonable radiation hardness due to their high degree of mineralization. Last but not least we would like to point out that this experiment greatly profited from the very helpful support by the beamline staff in preparation and during the beamtime itself.