

Elucidating the Crystallographic Microstructure of Human Tooth Enamel with Sub-micron Resolved Synchrotron X-ray Diffraction

R. Free¹, K.D. DeRocher¹, S.R. Stock², R. Xu³, D. Joester¹

¹Northwestern University, Department of Materials Science and Engineering, Evanston, IL, USA

²Northwestern University, Feinberg School of Medicine, Chicago, IL, USA

³Argonne National Lab, Advanced Photon Source, 34ID-E, Lemont, IL, USA

*Corresponding author contact: RFree@u.northwestern.edu

Tooth enamel, the outermost layer of human teeth, is a complex, hierarchically structured biocomposite. The details of this structure are important in multiple human health contexts, from understanding the progression of the infectious disease dental caries (tooth decay) to understanding the process of amelogenesis and related developmental defects. Many microstructural aspects of human enamel are qualitatively established (schematic in **panel A**): specifically, the nanostructure of hydroxylapatite (OHAp) crystallites elongated along their crystallographic c-axis with 20nm x 50nm rhombohedral cross-sections, bundling of $\sim 10^4$ crystallites into rods with $5\mu\text{m} \times 9\mu\text{m}$ keyhole-shaped cross-sections, and a less ordered interrod region where crystallites are less aligned and remnant organics are concentrated. Within each rod, the c-axes of the nanowires are roughly aligned to the rod axis, but they are thought to vary near the boundaries of the rod (**panel A**). The submicrometer transmission x-ray diffraction mapping results reported here are the first quantitative, 3D mapping of crystallite orientations.

Studies with TEM show the relationship between small groups of crystallites, and X-ray diffraction shows averages over $10^2 \mu\text{m}$, but direct measurement of varying local crystallographic structure across and between rods has been missing. Here, mapping with a $\sim 500\text{nm}$ diameter beam of monochromatic X-rays explores the spatial variation of crystallite orientations in human enamel. Several $1\mu\text{m}$ -thick sections were prepared and covered the 3 orthogonal tooth orientations shown in **panel A**. 2D diffraction maps of local crystallite populations were collected at an intermediate length scale that cannot be independently measured with low-resolution techniques like broad-beam X-ray diffraction nor practically sampled via high-resolution techniques like TEM.

Spatial diffraction maps recorded in 3 orthogonal orientations provide detailed sub-rod structural information. Each pattern in the map consists of incomplete diffraction rings reflecting texture and preferred orientation. In section 1, observed parallel to the rod axis, almost no $\{002\}$ reflections were observed, but key differences in the degree of azimuthal order was observed in the quadruplet reflections ($\{121\}$, $\{112\}$, $\{030\}$, $\{022\}$). By computing an azimuthal autocorrelation (**panel B and C**), these differences in crystallographic order could be quantified to distinguish rod and interrod sample points. High autocorrelation corresponds to rod regions, while lower autocorrelation marks interrod regions. In sections 2 and 3, c-axis orientation and spatial divergence are extracted by fitting the $\{002\}$ reflection (**panel D**), revealing the local variation of the crystallite orientation across multiple rods for the first time. Analysis of the integrated 1D diffraction patterns suggests there may also be local differences in crystallography at the submicrometer level. For example, lattice parameters within interrod regions appear to differ slightly from those in the rod (**panel E**). Variations across individual rods may also exist.

This novel experimental approach overcomes many of the previous challenges to enable systematic characterization of human enamel at an until now difficult to capture length scale. By filling in this gap, we aim to create a more complete picture of the enamel microstructure, informing efforts to understand the progression of dental caries and refine our understanding of the biomineralization processes involved in enamel formation.

