Experiment Brief

K3 IS Camera, STEMx System

Title

Electron counting 4D STEM studies of human tooth enamel

Gatan Instrument Used

The K3® IS camera delivers simultaneous low-dose imaging via real-time electron counting, fast continuous data capture, and a large field of view (FOV). In a 4D STEM experiment, the STEMx™ system precisely synchronizes the speed of the scanning probe to the camera frame rate to enable high-speed data acquisition and eliminates the potential for data loss.

Background

Dental enamel is the outer layer component of human teeth that comprises a complex, hierarchically-structured biocomposite. Impairment of an individual's dental enamel can dramatically affect their quality of life. Therefore, structural details are of high importance in multiple human health contexts. Enamel crystallites nominally contain the mineral apatite, which can be investigated using (scanning) transmission electron microscopy ((S)TEM). Sensitivity of this material to the electron beam is one of the limiting factors for performing such studies. Recently, atomic resolution STEM imaging successfully revealed the coherent atomic structure of crystallites in detail (Figure 1a). However, this required 1) cooling the sample to liquid nitrogen temperature and 2) using significant electron doses of ~6 x 10^3 $e^-/Å^2$ to achieve high signal-to-noise (SNR) images without sample decay. Additionally, 3) a relatively small FOV of the specimen with a limited number of crystallites could be captured per image.

Materials and Methods

A K3 IS camera and STEMx system were used to capture 4D STEM diffraction datasets at 300 frames per second in electron counting mode on a JEOL JEM-ARM 300F (S)TEM. The focused ion beam-prepared sample of outer tooth enamel was imaged at room temperature (in contrast to STEM imaging at cryogenic conditions). The full dataset was 512 x 512 x 262 x 266 pixels (132.5 x 132.5 nm^2), and it took ~240 s to acquire, with a total dose of only ~200 $e^-/Å^2$ (Figure 1b,c). DigitalMicrograph® software and Python scripting were used on an offline computer to process the binned-by-two data and create segmented virtual detectors (Figure 1c). Qualitative analysis of the difference in intensity between two opposing segmented detectors reveal that single crystallites display a tilt within the apatite lattice (Figure 1e,f). This suggests that enamel crystallites may not be as coherent as the cryo-STEM images imply, which in turn could have important implications on mechanical properties and provide unique insight into crystallite growth during enamel formation.

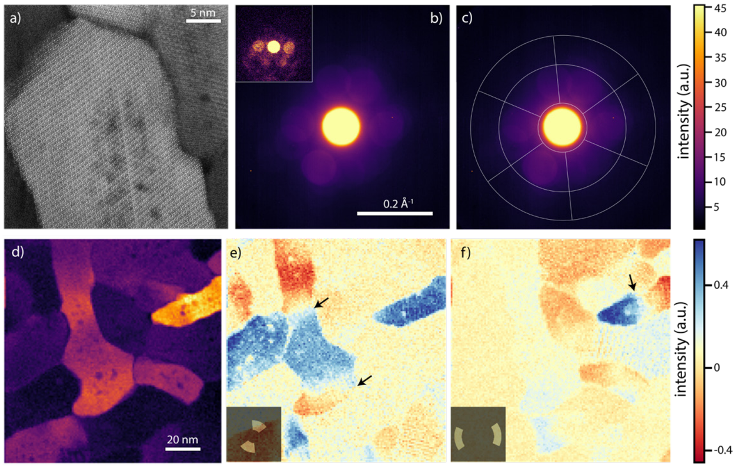

Figure 1. a) Cryo-STEM image displaying part of an enamel crystallite. Image adapted with permission from the below reference. b) Average diffraction pattern of the 4D STEM data set. Inset: Single diffraction pattern of the same dataset. c) Average diffraction pattern with 12 segmented virtual detectors (indicated by white lines). d) Virtual image created from the average diffraction pattern, combining intensities from all segmented virtual detectors. e,f) Maps displaying the differences in intensity between two selected opposite partial virtual detectors (indicated in inset). The black arrows indicate places within single grains that show variations in intensity between the opposed virtual detectors, which signify a tilt in the crystallographic directions of the apatite lattice with respect to one other.

Summary

The K3 IS with STEMx enables hardware synchronized 4D STEM diffraction experiments at very high speed (>3500 fps at 256 x 256-pixel resolution) and at the highest possible SNR (using electron counting). Thus, samples can be studied at lower dose conditions to minimize beam damage artifacts. Also, unlike HRSTEM, 4D STEM with the K3 IS allows studies of larger FOV (~12x in this case) and gives access to a broad range of angles in the reciprocal space that can be used to generate orientation maps and better characterize and understand such materials.

Credit(s)

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Professor Vinayak P. Dravid serves as the founding director of the NU*ANCE* center and SHyNE Resource, where this work was conducted.

Gatan, Inc. is the world's leading manufacturer of instrumentation and software used to enhance and extend electron microscopes—from specimen preparation and manipulation to imaging and analysis.

Reference: DeRocher, K.A., Smeets, P.J.M., Goodge, B.H. et al. Chemical gradients in human enamel crystallites. Nature 583, 66–71 (2020). https://doi.org/10.1038/s41586-020-2433-3

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