Atom Probe Tomography of Biological Apatites

Imaging Interfaces and Interphases in Tooth Enamel with LEAP

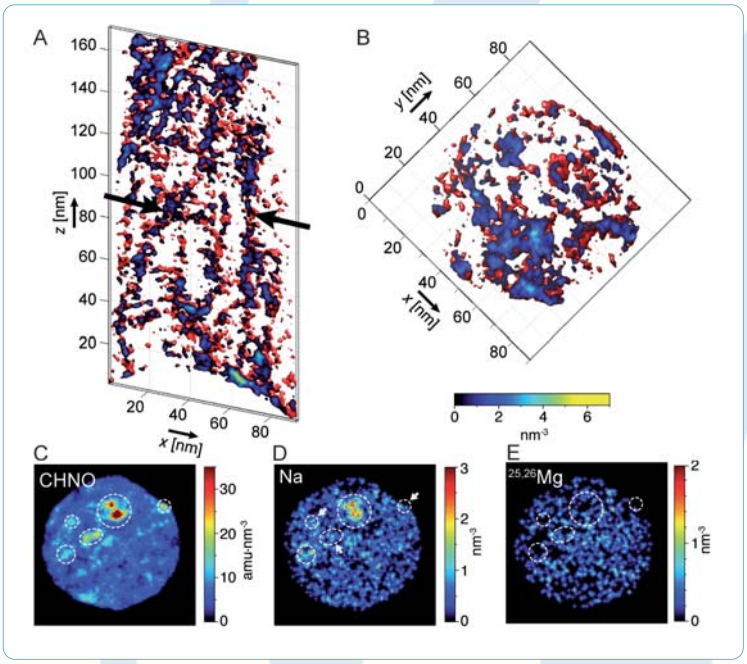
Application Note #APT-12

Nanocrystalline biological apatites $[Ca_5(PO_4)_3X, X = OH, F]$, often bearing additional impurities such as carbonate ions, constitute the mineral phase of vertebrate bone and teeth. Beyond their central importance to the mechanical function of our skeleton, their extraordinarily large surface acts as the most important ion exchanger for essential and toxic ions in our body. Nanoscale structural and chemical complexity of apatite-based tissues is a formidable challenge to quantitative imaging by electron, optical and X-ray methods. Atom probe tomography (APT), however, is uniquely suited to the task.

Bone and dentin are hierarchically structured materials with three major components: apatite (~72 dry wt%), organics (~20 dry wt%), and water. The mineral phase best resembles OH-deficient hydroxylapatite (OHAp) substituted with significant carbonate (5-8 wt%) and smaller levels of Na^+ and Mg^{2+} (0.5-1.0 wt%). The poorly crystalline mineral is thought to be present in irregular platelets approximately 50 x 25 x 2-5 nm^3 in size. Atom probe tomography enabled imaging organic-inorganic interfaces in elephant dentin (Figure 1). Remarkably, collagen fibers were found to show differential binding of sodium and magnesium. Counterintuitively, there was no indication of segregation of sodium, magnesium, or small organics to homophase (grain) boundaries.

Segregation was a defining feature, however, in rodent dental enamel (Figure 2). Enamel is comprised of apatite nanowires that are aligned along the crystallographic c-axis, and bundled into rods. Comparing 3D reconstructions of APT data from regular (Mg-substituted), and pigmented (iron substituted), enamel, segregation of Mg and Fe to grain boundaries is immediately apparent. In regular enamel, a Mg-rich intergranular phase was identified as Mg-substituted amorphous calcium phosphate. In pigmented enamel, Mg is replaced by Fe, and the amorphous intergranular phase is likely a Ca-substituted amorphous ferric phosphate.

Figure 1: 3D reconstruction of an APT data set from elephant dentin.



A, B: Rendering of slices showing an isoconcentration surface (0.7 ions nm^3, red) outlining organic material with isocaps indicating number density of organic fragments (colorbar).
C, D, E: Maps showing organic mass density, Na, and Mg number density of an x-y slice. Collagen fibers are indicated by black arrows (A, B) and white dashed circles (C-E).

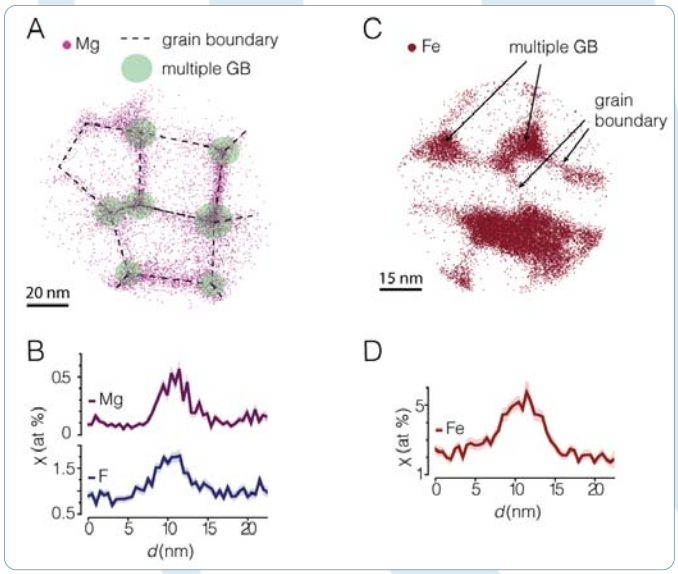


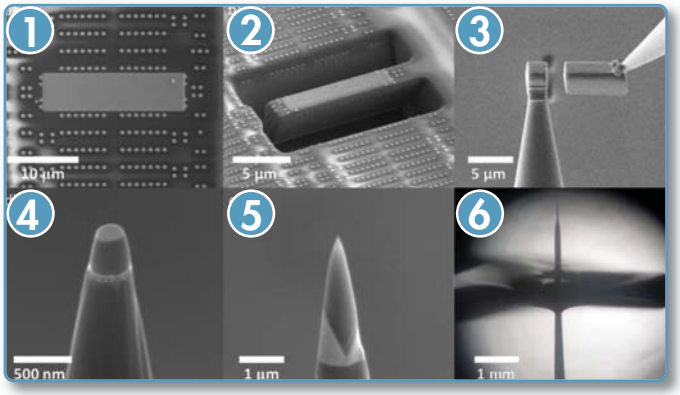
Figure 2: 3D reconstruction of regular mouse (A), and pigmented rat (C) enamel, and concentration profiles across grain boundaries showing Mg and F segregation in regular enamel (B) and Fe segregation in pigmented enamel (D).

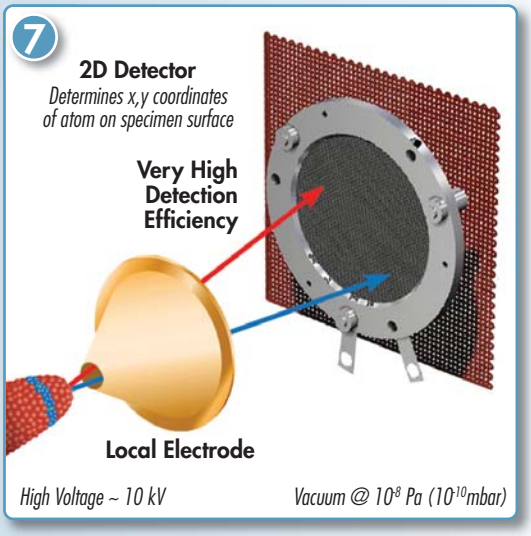
Adapted from L. M. Gordon et al., ACS nano 2012, 6, 10667-10675; L. M. Gordon et al., Science 2015, 347, 746-750; L. M. Gordon, Frontiers in Physiology 2015, 6

Three Steps to 3D Nanoscale Analysis
An Introduction to Atom Probe Tomography

Step 1: Specimen Preparation

An atom probe specimen usually has a nanoscale region of interest (ROI) requiring both 3D compositional imaging and analysis. The sample is formed into a needle shape containing the ROI. Common APT specimen preparation methods using electropolishing or a Focused Ion Beam system (FIB) are very similar to TEM methods except instead of forming a thin sheet, a needle shaped sample is desired. At the right, standard FIB liftout and mounting of a specimen (figures 1 through 3) and then sharpening the sample with the ROI left at the very apex (4 and 5). In 6, a wire geometry sample is being electropolished.





Step 2: Data Collection

An atom probe produces images by field evaporating atoms from a needle-shaped specimen and projecting the resultant ions onto a detector 7.

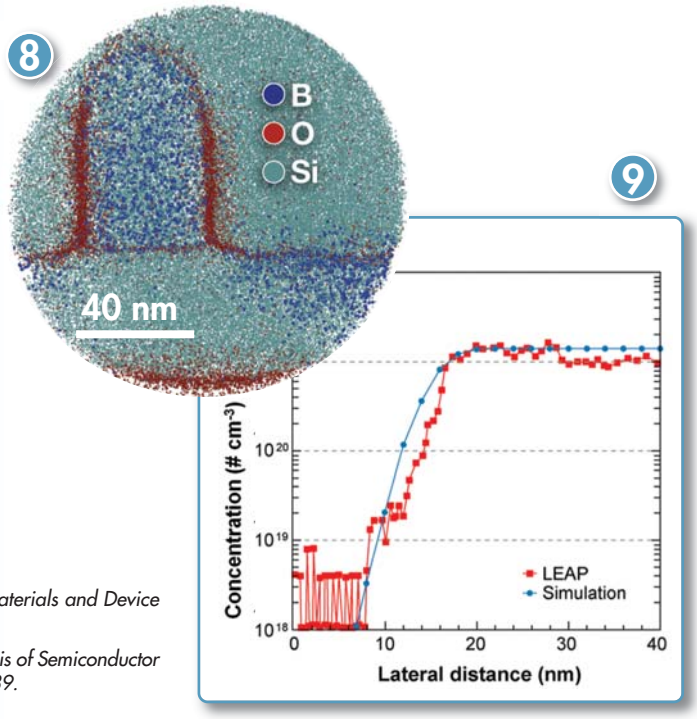
A high magnification results from the ~ 80nm tip being projected onto an 80mm detector resulting in a magnification of approximately 10^6.

An atom probe identifies atoms by their mass-to-charge-state ratio *(m/n)* using time-of-flight mass spectrometry. Charge state, *n*, is typically 1 to 3.

The specimen is held at approximately 50K to reduce surface diffusion during the experiment. The high electric field results in 100% ionization and the high speed detector is capable of measuring up to 80% of the collected ions, independent of ion mass.

Step 3: Data Visualization and Analysis

Examples of data output are illustrated by a slice of a 3D atom map of a transistor[†] 8, and a dopant composition profile[‡] 9. The image shows the positions of individual atoms (oxygen is red and boron is blue) in the transistor with subnanometer resolution. From the reconstructed data set many types of useful analyses are possible. These include 3D visualization, 2D atom mapping 8, 1D depth profiling and line scanning 9, as well as mass spectra and compositional analysis from user-selected volumes.



[†] *Lauhon, L. J. et al, MRS Bulletin "Atom Probe Tomography of Semiconductor Materials and Device Structures" 34(10) (2009) 738.*

[‡] *Moore, J. S.; Jones, K. S.; Kennel, H.; Corcoran, S., Ultramicroscopy "3-D Analysis of Semiconductor Dopant Distributions in a Patterned Structure using LEAP" (2008), 108, 536–539.*

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