MINERALIZED TISSUE FORMATION DESCRIBED BY SYNCHROTRON-BASED X-RAY ANALYSIS AND IMAGING TECHNIQUES

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Living organisms form complex mineralized composite materials that perform a variety of essential functions, ranging from structural support and mechanical strength, to optical, magnetic or sensing capabilities. This remarkable diversity in functionality is accomplished from a relatively narrow range of constituent inorganic materials via hierarchical mineral-organic functional architectures. Therefore, these structures routinely serve as a source of inspiration for scientists and engineers. The control over biomineral shape, at all hierarchical levels, is a key aspect of structure-to-function relationship in biological materials. Although many studies have emphasized the critical role of biological regulation during biomineral formation, the physical constraints governing the growth process of naturally occurring architectures and determining the form of biomineral building blocks are not understood.

In this talk, I will address the fundamental question of how nature takes advantage of thermodynamic principles to generate complex morphologies. I will highlight two structures where, using synchrotron-based X-ray analysis and imaging techniques, it was shown that the micro-structure formation process during biomineralization is analytically defined and can be quantitatively described both in time and in space. The structures are the prismatic layer in the shell of a bivalve mollusc Pinna nobilis (1) and the anchor spicule of a glass sponge Monorhaphis chuni (2, 3).


DARK-FIELD HARD X-RAY MICROSCOPY IMAGING FOR THE STUDY OF BIOMINERALS

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A full-field Hard X-ray Microscope (HXRM) has been constructed at ESRF ID06 for the study of polycrystalline materials. Used in dark-field mode, the Bragg diffracted beam produced by a given grain is selected and magnified using a stack of Be compound refractive lenses. The resulting image, a real space projection of the grain, is captured by an imaging detector. Coupled to a high-precision goniometer, this permits characterisation of orientation and mosaicity with a real space resolution of 100 nm and angular resolution of 10 mrad. Strain within a grain can be measured with a resolution of 10⁻⁵. A single projection can be recorded in one second, and mosaicity maps can be recorded in a few hours, providing the possibility for studies of dynamics. The microscope has so far been used for characterisation of metal and ferroelectric samples, and work is underway to expand the technique to new areas.

One such example is the study of biominerals, biological hierarchical materials combining an organic template with a mineral structural component. Biominerals exhibit a wide variety of functions, with forms which are controlled by the animal’s physiology. Their microstructure is often tailored to provide particular material properties, making them interesting as natural models for the inspiration of new engineered materials.

We will show results from our initial examinations of pearl, bivalve shell, and fish otoliths, highlighting some of the challenges in their analysis and the potential of dark-field HXRM to reveal structures on scales from...
micrometric to nanometric in these and other complex polycrystalline materials.


Iron is a primary component of fundamental processes which can become toxic when present in excess. In human fluids, free iron is maintained at 10-18M concentration thanks to several proteins as lactoferrin (Lf) in secretions and transferrin in blood. The altered iron balance favors bacterial infection and the related inflammatory response as occurs in cystic fibrosis [1, 2]. Therefore, it is of great importance to provide quantitative mapping of iron concentration at high spatial resolution. Here we studied human phagocytic cells unstimulated or stimulated with bacterial lipopolysaccharide (LPS) or and Lf to map the intracellular density and iron concentration. For this aim, X-ray fluorescence microscopy (XRFM), atomic force microscopy (AFM) and phase contrast imaging were combined, as previously demonstrated [3, 4]. To determine the concentration map, we normalized the fluorescence intensity with the volume of the illuminated region Fig.1. The volume of freeze-dried cells has been obtained by AFM with lateral resolution of 100 nm and 50 nm, respectively. Moreover, we determined the weight fraction distribution map, normalizing the fluorescence intensity with the projected density obtained by phase contrast imaging Fig.2 [5]. Indeed, we obtained the density distribution Fig.3 by normalizing phase reconstruction maps with AFM data. Similar evaluations were carried out for Lf- and LPS plus Lf-treated cells. We also carried out nanotomography measurements, to obtain the three-dimensional density distribution Fig.4. Information about the electron density combined with the average density of the sample allows to calculate its thickness, value which can be compared with alternative techniques AFM. The nanotomography is of paramount importance to reach the volumetric information in frozen-hydrated cells because AFM cannot be used since frozen hydrated cells are stored in liquid nitrogen.

2. P. Valenti et al., Lactoferrin and cystic fibrosis airway infection. In: Diet and Exercise in Cystic Fibrosis. 2015, 30, 259.


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TOWARDS DOSE EFFICIENT IN-VIVO X-RAY MICROSCOPY OF BIOLOGICAL SYSTEMS USING BRAGG MAGNIFIER MICROSCOPE

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In this work we present the actual status of the development of Bragg Magnifier Microscope based on in-line Germanium crystals [1, 2] with the focus on the improved phase retrieval algorithm. This imaging system is using asymmetric Bragg reflections to geometrically magnify X-ray beam up to 250 times and the beam is directly detected by single photon counting detector. We performed successful testing of this imaging system at Diamond Light Source I13 and B16 and at Spring 8 radiation facilities and applied it for full field, single distance, holographic imaging of biological samples. The improved phase retrieval algorithm [3] takes advantage from the combination of the modified shrink-wrap algorithm for phase objects, robust unwrap-ping algorithm as well as other reasonable constraints applied to the wavefield at the object and the detector plane. The spatial resolution, achieved after successful phase retrieval of recorded holograms (Fig. 1), was ~300 nm and the acquisition time for one frame at ID13 was 0.3s, which can be still minimized to millisecond region. In diffraction limited sources the throughput of the device will be maximized due to matching divergences, which will bring additional exposure time decrease. According to achieved state of the development: high resolution, short acquisition time, single distance phase retrieval, we propose this system for in-vivo 2D/3D quantitative imaging of biological samples such as cells or small animals. In this work we will summarize recent state of the development, introduce single distance phase retrieval method and demonstrate it on successful 3D reconstruction of biological organism (Tardigrade).


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