	Experiment title: Mesostructure evolution the of prismatic layers in marine shells	**Experiment number**: SC-4046
Beamline: ID19	**Date of experiment**: from: 30.04.2015 to: 04.05.2015	**Date of report**: *Received at ESRF:*
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Report:

Background

This beam time was applied to investigate the prismatic layer of several mollusk shells, which will contribute to the understanding of the thermodynamic aspects involved in the biomineralization process of these biological structures. The prismatic layer, representing the larger part of the shells, is a hierarchical biocomposites that consists of elongated stiff calcite prisms surrounded by a softer organic phase. Previous experiments on ID19 allowed us to resolve the shape of individual prisms in the prismatic layer in *Pinna nobilis* (SC-3726). There, we could show that the mesostructure formation can be described using classical theories for normal grain growth and coarsening, suggesting that the organism forms specific boundary conditions to promote the growth of a specific morphology[1]. The prismatic layers in different shells exhibit different morphological properties and the question is whether different boundary conditions are formed to promote the development of this variety of structures and what are they?

Experiments and Setup at ID19

Samples of the mollusc species *Atrina rigida, Atrina vexilum, Atrina histrix, Unio pictorum, Pinctada margaritifera, Pinctada nigra, Pinctada radiate and Inceramus* were investigated. In each case three cubic blocks were cut out of the prismatic layer of each species and polished to 15 cylindrically shaped specimens with a diameter of ~1mm.

The 3D spatial arrangements of these sample sets were imaged by synchrotron radiation computed microtomography (SR µCT) at ID19. The samples were scanned with X-ray energies between 19 and 35 keV. More than 2000 radiographic projection images were recorded over 180 degrees with different exposure times

[1] Bayerlein, B., Zaslansky, P., Dauphin, Y., Rack, A., Fratzl, P., & Zlotnikov, I. (2014). Self-similar mesostructure evolution of the growing mollusc shell reminiscent of thermodynamically driven grain growth. Nature Materials, 13(October), 1102–1107. http://doi.org/10.1038/NMAT4110

ranging from 0.3 to 1 s. Distances were varied for absorption- and phase-contrast enhanced imaging modes. A mulitlayer monochromator was used to obtain higher photon flux density to increase the contrast and the resolution of the scans. Through superposition and phase-retrieval of the images obtained at different distances, holotomographic imaging will be possible. Pixel sizes of 648 nm were obtained.

Analysis and Results

Due to the high quality µCT measurements at ID19 it was possible to resolve the organic-calcite assembly of the prismatic layer of all investigated species. Figure 1 shows some reconstructed 2D slices of three exemplary species, which demonstrate the diversity in the morphology of the prisms. According to the information received from these data, we expect to obtain a novel and a comprehensive insight into the biomineralization process and the structural evolution of the prisms. To ensure statistical accuracy, high numbers of prisms need to be analyzed. Therefore, automated prism segmentation using a watershed algorithm is used (Figure 2). Image processing and data analysis is still in process but preliminary data from *Pinctada margaritifera* suggest a linear growth behavior of this species (Figure 3) similar to what was published for *Pinna nobilis*.

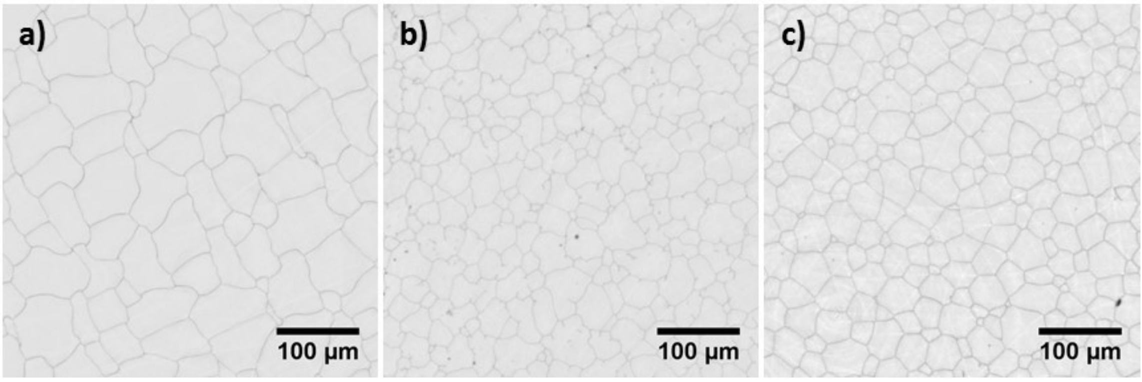

Figure 1: Paganin reconstructed images of a) *Pinctada nigra*, b), *Atrina histrix* and c) *Pinctada margaritifera*

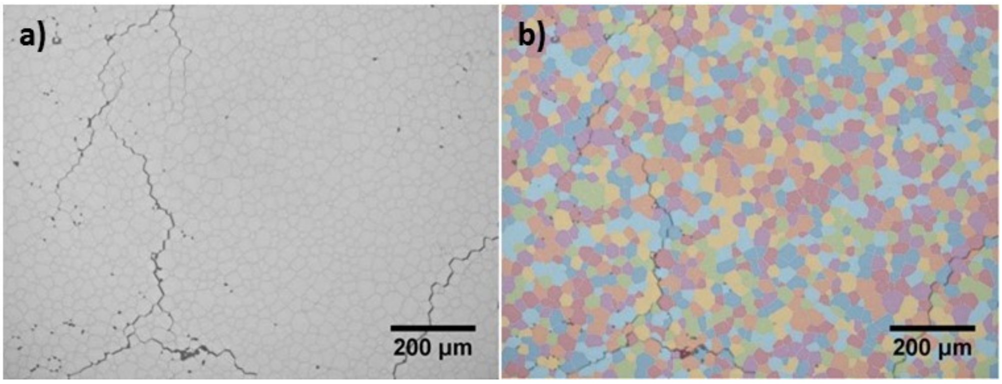

Figure 2: Automated prism segmentation of *Pinctada margaritifera;* a) original reconstruction image; b) watershed segmentation of the prisms

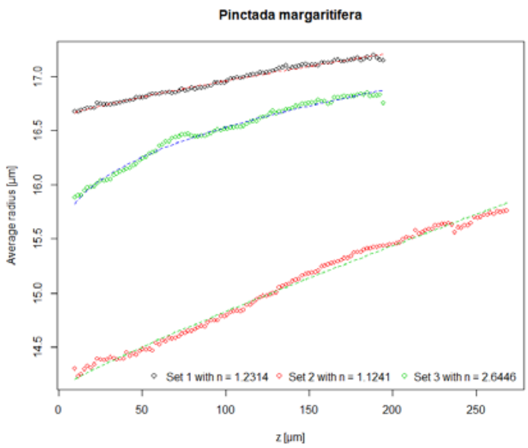

Figure 3: The average prism radius of *Pinctada margaritifera* was plotted against the growth direction and fitted with the equation $R = Kt^{1/n}$ (R: average radius; K: a constant proportional to the energy and mobility of the boundary; n = grain growth exponent; t: time/growth direction)