EUROPEAN SYNCHROTRON RADIATION FACILITY

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ESRF	**Experiment title:** Nanoscale reticulation of calcareous biocrystals investigated by 3D Bragg ptychography	**Experiment number**: SC3550
Beamline: ID13	**Date of experiment**: from: 28/03/13 to: 05/04/13	**Date of report**: 18/09/13
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Report:

The SC3550 experiment aimed at demonstrating the possibility to use Bragg Ptychography to explore the still mysterious and generic mechanisms of bio-crystallization. Indeed, calcareous structures produced by living organisms exhibit species-specific morphologies at the macro and micro-scales. However, the mineralized unit building blocks present a single crystalline behavior, together with a generic granular topological structure as observed with AFM [1]. Diffraction pattern obtained by TEM confirms the identical orientations of the grains within a given microstructural unit. This tends to indicate that within a growth layer, organic and mineral components form a reticulate structure, resulting from the growth process. However, only a three-dimensional description of the crystalline part within a nanoscale resolution would give the information needed to validate the specific crystallization process. In this context, we used Bragg ptychography as a technique able to provide a quantitative 3D image of the crystalline structure with a resolution in the tenth of nanometer range. Instead of lens, inversion algorithms are used to retrieve the image of the sample. First demonstration of this lens-less imaging technique has been published by some of the present applicants [2].

During our experimental session at the ID13 beamline, we acquired x-ray Bragg ptychography data to investigate the internal structure of the calcite biominerals composing a *Pinctada margaritifera* shells. Special care was taken for the sample preparation: a small part of the shell edge was selected, in order to ensure that only a few (ideally a single) growth layers would be investigated at the same time.

The experiment took place at the 100 m long beamline (EH3). The focused spot was produced by the monochromatic (λ = 0.8Å) and coherent illumination of a set of refractive lenses. The typical size of the FWHM intensity spot was estimated to 100 nm. Prior to the sample investigation, the direct beam illumination was investigated in details with a PCO camera (pixel size 1.88 μm) located at 1.8 m from the focusing optics (Fig. 1a). This allowed to retrieve the complex-valued wave-front illumination in the focal plane (Fig. 1b,c).

The sample, placed on a piezoelectric 3D translation stage on an hexapod, was accurately aligned in the focal plane using the dedicated optical microscope. The transmission geometry was chosen in order to optimize the beam footprint onto the sample. In addition, we ensured the selection of the thinnest part of the sample (about

1 μm), very close to the native shell border. This thickness allows to preserve the coherent interferences along the beam path through the sample.
The Bragg conditions were found by scanning the sample in the vicinity of the Bragg angle (θ_B = 10°) and measuring the diffraction pattern with the 10 × 10 cm^2 Frelon camera located close to the sample. Finally, the Maxipix detector was used at a 2.5 m distance to measure the coherent Bragg diffraction pattern, in the horizontal plane at the 110 and 012 reflections. The full 3D intensity distribution was obtained by scanning the sample along the rocking curve. A total of three sets of Bragg ptychography measurements could be obtained either for different positions of the beam spot onto the same mineralized unit or for different samples. **These data sets exhibit enough contrast, signal to noise ratio, overlapping and diversity in order to allow for a successful phase retrieval process. This is done by our 3D Bragg ptychography algorithm, which allows us to observe for the first time the internal structure of the shell, in situ and in 3D, with a resolution of about 10 × 10 × 10 nm^3.** The retrieved direct space 3D phase map (Fig. 2b,c) holds the information concerning the material crystalline properties (coherence, orientation, etc.). This reconstruction confirms our previous observation: the calcite biocrystal can be considered as a single crystal only to some extent. The typical size of the single crystal domain is about 200 nm, laterally. Interestingly, the crystalline coherence is mostly preserved along the growth direction.

Finally, Bragg nano-diffraction maps were also measured, in order to explore the presence of crystalline material in the early stage of the shell growth. An isolated prism (namely, a disk) was selected and the corresponding diffraction patterns were acquired with the large field of view (Frelon) camera. These preliminary measurement shows that the crystalline material is already present in the isolated disk, with however some internal spatial variation. No evidence of crystalline material has been observed in the disk external surrounding, so far.
As this was clear that the coherent nano-beam set-up was not optimized to perform and exhaustive Bragg nano-diffraction investigation of shell, we decided to apply for a dedicated proposal. Indeed this study requires a specific optimized (incoherent) set-up in order to bring the decisive information allowing for the understanding of the shell early stage growth.

References

[1] Y. Dauphin, J. Biol. Chem. **278**, 15138 (2003); Y. Dauphin, Mineral. Mag. **71**, 247 (2008).
[2] P. Godard *et al.*, Nature Comm. **2**, 568 (2011).

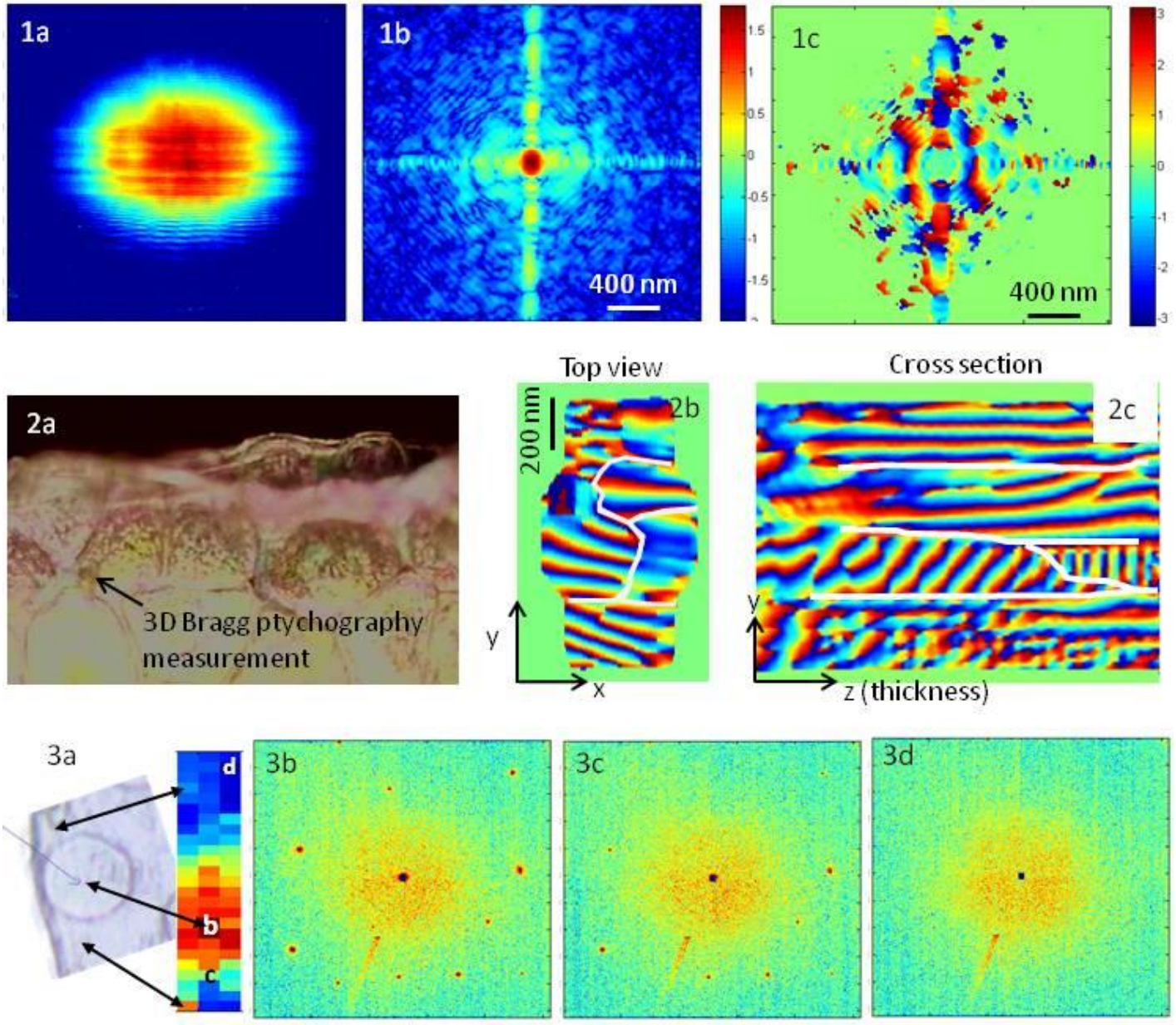

Figure 1: (a) Overfocused direct beam intensity measured in the far-field regime for a coherent illumination of the focusing optics. (b) and (c) Reconstruction of the illumination function at the sample position obtained directly from (a): amplitude and phase, respectively.

Figure 2: (a) Optical view of the *Pinctada margaritifera* shell investigated by 3D Bragg ptychography. (b) and (c) Reconstruction of the phase distribution within the shell, obtained by the inversion of the 3D intensity patterns. The iso-phase ramp area are resulting from the existence of single crystalline domains. Each domain have a slightly different orientations.

Figure 3: (a) Preliminary Bragg micro-diffraction investigation of an isolated prism (disk), corresponding to the early stage of the shell growth. The colored map represents the integrated intensity of the observed Bragg peaks. (b), (c) and (d) Bragg diffraction patterns collected at different positions of the disk: center, border and outside, respectively.