EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

	Experiment title:	**Experiment number**:
ESRF	Nanoscale reticulation of calcareous biocrystals investigated by 3D Bragg ptychography	SC3065
Beamline: ID13	**Date of experiment**: from: 16/02/11 to: 24/02/11	**Date of report**: 27/02/12
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Report:

The SC3065 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 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 proposed to use 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 recently 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: as the growth consists in the superposition of synchronous growth-layers of about 2 μm thick, 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. This is of particular importance to avoid lack of x-ray coherence in the detection scheme.

The experiment took place at the 100 m long beamline (EH3). The focused spot, produced by the monochromatic illumination (λ = 1Å) of a Fresnel Zone Plate (FZP), was made coherent by reducing the optics aperture down to the transverse coherent length of the beam. A central spot at the FZP and an order sorting aperture located closed to the sample were used to avoid the direct beam contribution and the higher diffraction orders, respectively. The sample, placed on a piezoelectric 3D translation stage on an hexapod, was accurately aligned in the FZP focal plane using the dedicated optical microscope. The 006 Bragg

reflection was considered for its favorable orientation, perpendicular to the sample surface within a ±10° angular distribution.

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 m distance to measure the coherent Bragg diffraction pattern, in the horizontal plane. The full 3D intensity distribution was obtained by scanning the sample along the rocking curve. A total of three sets of Bragg ptychography measurements were obtained either for different positions of the beam spot onto the same mineralized unit or for different samples. Figure 1(a,b) shows different coherent intensity patterns taken at different translational positions and different rocking angles. The preliminary analysis of these data show a high data quality compatible with the ptychography inversion scheme. This results from the high stability of the ID13 nano-setup and the coherence properties of the beam (up to 70 % speckle visibility was observed). In addition, the observed speckle distribution and the extend of the Bragg peak show already the limits of the single crystal model, which is no more valid at least to some extent. Several micro-diffraction maps were also measured over area as large as the mineralized unit (figure 1(c)). They will be used to measure the crystal orientation and orientation distribution variation. We expect that the forthcoming phase retrieval of the ptychography data will allow to image the relative orientation of the nano-grains within the crystal. However, our analysis might be limited by the slightly too small sampling rate.

Due to the novelty of this kind of experiment at the ID13 beamline, we spent about 10 shifts for the setting of the optics, the diffraction stage and several 2D detectors. Extended characterizations of the experiment coherence properties were performed for different FZP aperture values, with and without the use of pre-focusing Be lens: we measured the direct beam overfocused far-field intensity pattern, with a high resolution detector (figure 2(a)). These patterns were analyzed using home-made inversion procedures based on propagation in the Fresnel regime. It allowed us to check in details the beam quality and to back propagate the field at the sample position (figure 2(b,c)). Furthermore for this pioneering experiment, we performed a Bragg ptychography experiment on a Si test object (using the 004 reflection, figure 3(a,b)). These data were inverted using our home-made developed Bragg ptychography inversion scheme using the knowledge of the previously imaged illumination function (figure 3(c,d)). The agreement between the sample and the 3D reconstruction demonstrates the possibility to perform Bragg ptychography at the ID13 beamline [3]. The conclusions brought by these coherence tests allowed us to accurately define the conditions for which the biomineral ptychography data were acquired.

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).
[3] V. Chamard *et al.*, manuscript under preparation.

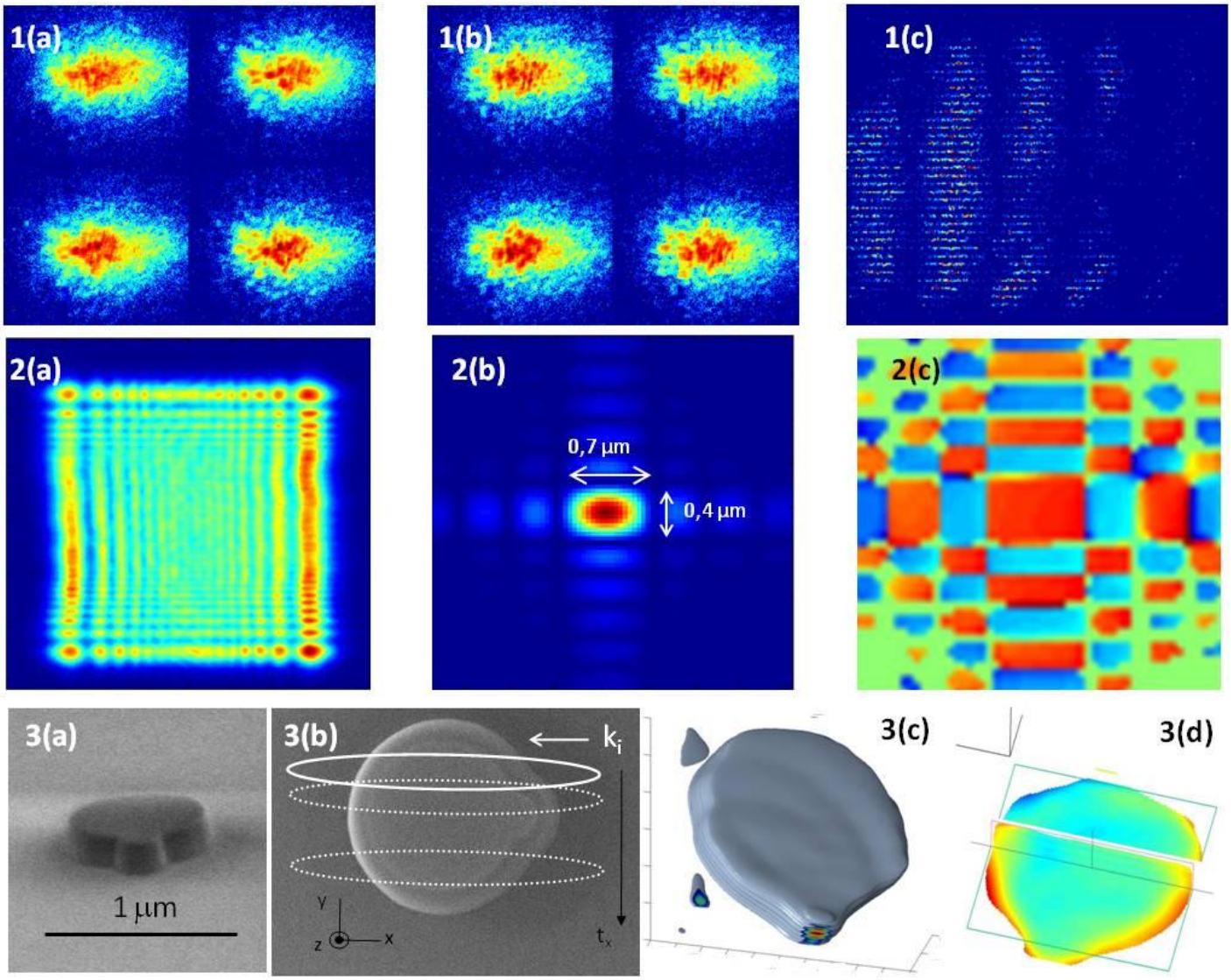

Figure 1: Coherent Bragg diffraction intensity pattern measured on the *Pinctada margaritifera* shell for different translational position at (a) $\theta_i = \theta_B$ and (b) $\theta_i = \theta_B - 0.02°$. (c) Bragg micro-diffraction set of data acquired on a complete mineralized unit.

Figure 2: (a) Overfocused direct beam intensity measured in the far-field regime for a partial illumination of the FZP. (b) and (c) Reconstruction of the illumination function at the sample position obtained directly from (a).

Figure 3: Preliminary Bragg ptychography tests were performed on a model Silicon sample. (a, b) SEM views of the sample and experimental geometry: the illumination footprint is represented by a white ellipse while the translation ptychography scan is indicated by t_x. (c) Iso-surface of the density distribution retrieved from the Bragg ptychography set of data and (d) internal phase of the sample showing the absence of displacement field in the sample almost everywhere except at the edges. The resolution of the reconstruction is slightly better than $20 \times 40 \times 60$ nm^3.