	Experiment title: Biomineral formation mechanisms in Pinctada margaritifera: a perturbation-based proteomic/Bragg ptychography approach	**Experiment number**: EV-394
Beamline: ID13	**Date of experiment**: from: 11.11.2020 to: 15.11.2020	**Date of report**:
Shifts: 9	**Local contact(s)**: Peng Li	*Received at ESRF:*

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Report:

Summary:

The initial aim of this proposal was the structural characterization of calcarous mollusc shells of Pinctada margaritifera to unveil the biological, physical and chemical pathways involved in the biomineralization following a perturbation approach. Here the mollusc shells are raised under sub-optimal pH and T conditions which modifies the mineralization mechanism.

The samples are grown in Tahit in collaboration with IFREMER, the french marine research institute. Due to the pandemic situtation in 2020, we were not able to obtain samples of sufficient quality and we thus had to switch our experimental approach on a synthetic crystallisation model that mimics important nanostructural aspects of the mollusc shell structure. This experiment is part of our overall biominalization project and was extremelly usefull to prepare exp. SC5117.

The aim of this study was to characterize and understand the solid/solid phase transformation mechanisms occuring during the non-classical crystallization of calcite films under organic mediation. Theory describes two possible mechanisms for the amoprhous-to-crystal transformation (solid-solid vs dissolution-reprecipitation). Our starting hypothesis was that the in-vitro model allowed to chose one of the two routes by tuning temperature and humidity during the crystallization.

We investigated samples from each crystallization route via nanodiffraction and 3D Bragg ptychography (3DBP) and could evidence strong differences in the shape and nanostructural organization of the films, confirming the two different transformation routes.

Samples and setup:

The sample set comprised two Calcite films, which were transformed from a similar amorphous calcium carbonate film under different crystallisation conditions (Temperature and humidity). While the starting amorphous films shows a very uniform, disc shaped morphology, the resulting crystalline films show either a preserve disc-like shape macroscopic morphology or a volute-like morpholology (see Fig 1)

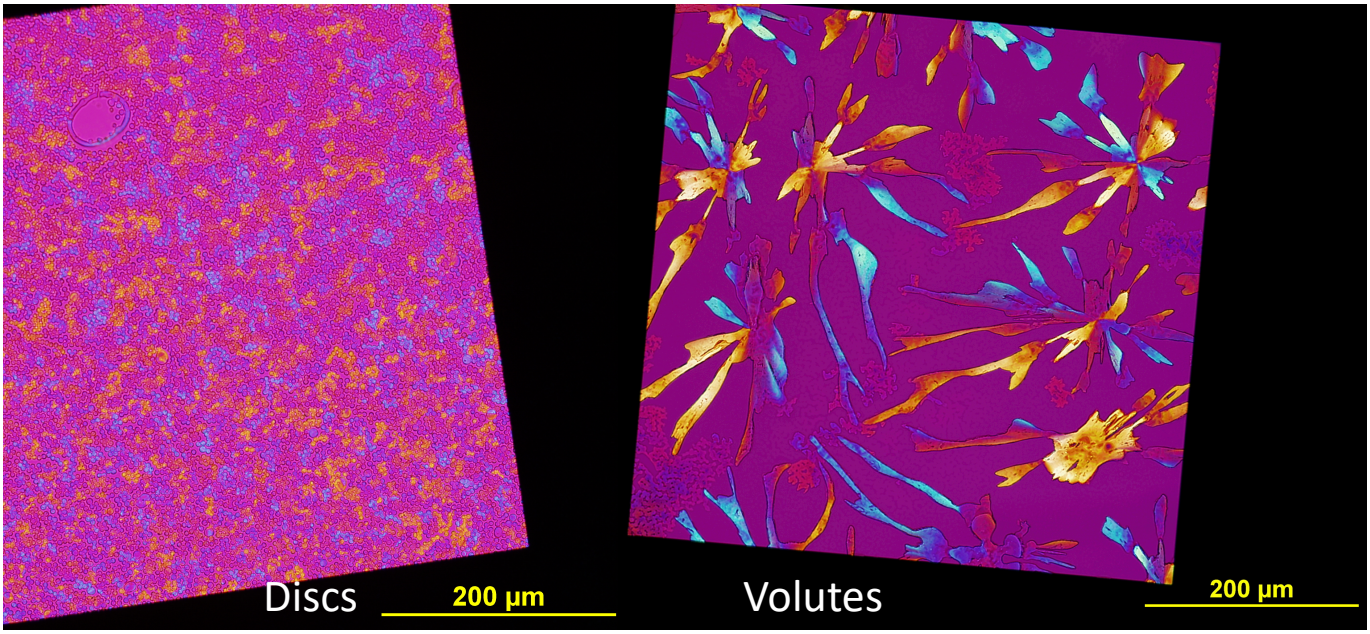

Figure 1 Comparision of two crystalline calcite films, obtained from similar amorphous films but with varying crystallization conditions resulting in disc-like and volute-like morphology.

Experiments were carried in the nanobranch of ID13 (EH3), using a set of silicon NFLs to produce a beam of ~250x250 nm^2 with a flux of 9 10^{10} ph/s at 15.2 keV photon energy. The diffraction signal was collected with an Eiger 4M for nanodiffraction experiments and a Maxipix for 3DBP experiements. In addition, the fluoresence signal of the samples was collected with a Vortex EM detector.
The experimental approach comprised the collection of rocking curve / nanodiffraction maps of each sample to characterize a statistically relevant area of the samples and to obtain strain information with beam-size limited resolution. These nanodiffraction maps further allowed to target isolated Bragg peaks in the horizontal plane for further 3DBP investigations.

Principal outcome:
We succesfully managed to collect complete nanodiffraction and 3DBP datasets on the two samples. As a side note, we want to point out that the increased coherence due to the ESRF-EBS upgrade together with improvements of the beamline instrumentation and the BLISS control system allowed us to significantly increase our data acqusition speed and resulted in largely improved data quality.

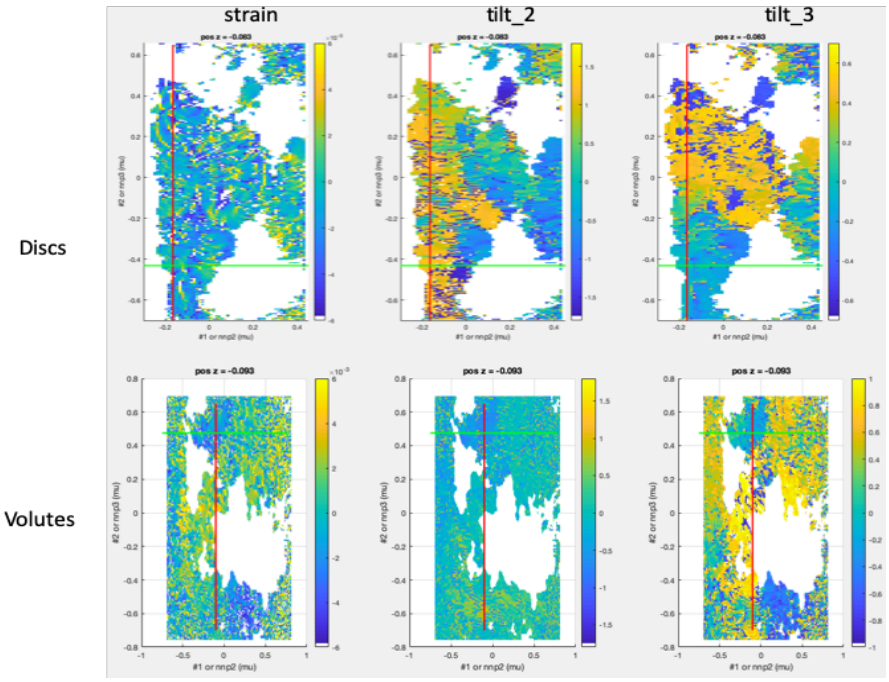

Figure 2 3DP reconstruction of Disc and Volute-like sampes.

The full data set was carefully analysed and the inversion of the 3D data was obtained. Figure 2 shows 3DBP reconstruction of the two samples. The disc sample exhibits iso-oriented domains (similar colour in tilt 2 and tilt 3) with a size of about ~150-200 nm and while the strain is rather homogenous, it shows some rather abrupt discontinuities. The domains in themselved are rather strain and distortion free and only show strain accumulation at the interface between two domains.
In contrast to this, the volutes, while also presenting iso-oriented domains, they appear much larger (300-500 nm size), more sinuous and span the entire thickness of the investigated volume. The strain is not as homogenously distributed as in the disc sample and shows up rather inside of the domains. Large strains are associated to phase vortices, which are signatures of the presences of (rather complex) dislocations.
The discs thus present a grain-like structure with strain-free grains. The grains are aligning with each other with a small rotation but without crystalline coherence propagating to neighbouring grains. The grain interfaces show increased strain. In summary, these observations fit well with a solid/solid phase transformation.
The volutes present rather large iso-oriented domains with crystalline coherence propagating through them. The internal structure of the grains is marked by distortions and strain, which are released at dislocations. This hints strongly towards atomic mobility as it would be possible in a dissolution/re-precipitaiton phase transformation.
A careful bibliographic work carried out at the moment, allowing us to better understand the link between the retreived phase field and the details of the two crystallisation mechanisms.

Conclusions and further proceedings:
In summary, we could evidence stark differences in the crystalline properties of the two calcite films and find them to be in agreement with anticipated solid/solid transition mechanisms. It shows the overriding importance of the crystallization in the final crystalline properties as the amorphous films were of similar structure. In the time since this experiment, we carried out further experiments on different crystallisation routes, which extends the understanding we have developped based on this experiments.
A publication which reports these results is in preparation and we anticipate to use the information gained from this experiment to be applied to other biomineral systems.
We would like to underline the great deal of support we received from the staff of the ESRF and ID13 in particular during this experiment.