	Experiment title: One cell to make them all – Elucidating the origin of polymorphism and micro-structure diversity in biomineralization from pearl investigation	**Experiment number**: EV 480
Beamline: ID13	**Date of experiment**: from: 21.06.2022 to: 27.06.2022	**Date of report**: 08.09.22
Shifts: 12	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

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Report:

Summary:

The aim of this experiment was the understanding of physico-chemical mechanisms at play during the biomineralization process of the pearl produced by Pinctada margaritifera. Pearls lend themselves as a model system for this experiment as they express complex biomineralized structures (several $CaCO_3$ polymorphs as well as different microstructural organizaiton schemes) in the presence of only a limited, single, cellular environrement, the pearl sac. This structure produces a constant set of organic molecules and control the physico-chemical parameters of the biomineralizing medium. The original proposal was based on an already established sample set which has been already investigated by ptychograpic tomography, and yieled the 3D electron density distribution. The present experiment aimed at investigating the presence of several crystalline polymorphs by diffraction tomography and a further study of selected sites during the growth with 3D Bragg ptychography.

The actual experiment was restricted to the 3D diffraction tomography experiments due to time lost during extended periods of beam loss. We were able to collect five full diffraction tomography datasets which shall give us insights into the polymorphic development of pearls and finally create the link between the crystalline polymorph and the microstructrural unit

Samples and setup:

The experiment was carried out at the ID13 EH3 nanobeam setup. The x-ray energy was set to 13 keV and a set of MLL lenses produced a spot of ~70x70 nm^2 with a flux of $5x10^{11}$ ph/s. The Eiger 4M detector was used to detect the scattering signal in a q-range of 0.5 to 35 nm^{-1} and the XRF signal was detected in addition. The sample was scanned with the PI Hera nanopositioner and a Smaract SR 2013 rotation stage was placed on top of the scanner to be able to rotate the sample while retaining the scanning coordinate system. Adapters to mount the OMNY pins employed at the SLS where produced by the users and left at the beamline for future use by other user groups.

The sample set comprised two samples, one sample of mature nacre and one sample which shows the full developmental sequence from the nucleation event to fully developped nacre (Fig 1 a), where all zones of interest like the transition zone (Fig 1b) and the nucleation zone (Fig 1c) are present. The samples were

produced by FIB milling and were mounted on OMNY pins. This sample set provides a full description of the development stages and was already measured with ptychograpic tomography to determine the electron density qualitatively. The diffraction tomography data was obtained with lateral step size of 100 nm and a rotational stepsize matched to about 1.5 D/r where D is the sample diameter and r is the target resolution (step size in this case). The angular sampling was carried out in 4 interlaced subtomograms to be able to detect and mitigate the onset of radiation damage. The data reconstruction was carried out with home-written scripts and an alignment procedure based on the tomographic self-constency alignment developed by the cSAXS team at the SLS. The data was aligned based on the SAXS signal, assuming rotational invariance of the signal and further reconstructed for the full q-range with a filtered backprojection approach.

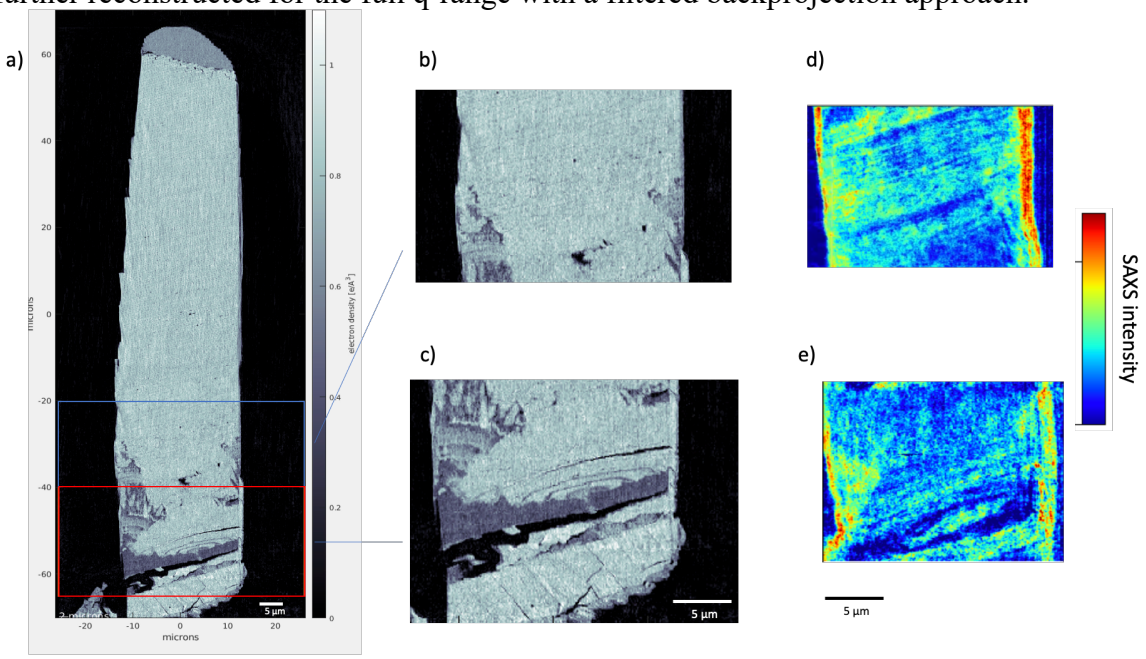

Figure 1 Forming nacre sample a) Full 3D ptychographic tomography reconstruction b) 3D ptycho tomo zoom-in on the transition zon c) 3D ptycho tomo zoom-in on the nucleation zone d) 3D diffraction tomography, reconstruction of the interface zone e) 3D diffraction tomography, reconstruction of the nucleation zone

Principal outcome:

Figure 1d) shows a cross sectin through the 3D reconstruction (SAXS intensity) of the interface zone, Fig 1e) shows the cross section through 3D reconstruction of the SAXS signal of the nucleation zone. The analysis was split into groups of subtomograms as the onset of radiation damage was detected towards the 3rd and 4th subtomogram (depending on the sample). This finding will give us an important indicator for future experiments how much dose can be deposited on the sample. It is also important to bear in mind that the samples have already been subjected to a full ptychographic tomography experiment, thus a crystallization of present amorphous calcium carbonate is expected and further radiation induced recrystallization of Aragonite into Calcite needs to be monitored carefully.

Due to the relatively recent nature of the experiment (End of June 2022), the data treatment is not fully finished yet, but we have verified the quality of all datasets and have aligned and inverted the SAXS signal of all tomograms. Further analysis of the full q-range is pending along with a more detailed determination of crystallographic parameters such as strain and Scherrer width along with a determination of the nanostructural parameters from SAXS.

Conclusions and further proceedings:

In conclusion, the major part experiment was carried out succesfully and only the part of 3D Bragg ptychography needed to be skipped due to time constraints arising from the beam loss. We are however confident that we have already gained crucial insights with the present diffraction tomography dataset. The complete data analysis is still pending, but the quality of the data has been already verified and we will soon prepare a publication to report our findings together with the earlier ptychograpic tomography experiments. From a techincal perspective, this experiment has also been used to greatly facilitate the implementation of 3D diffraction tomography experiments and will be of great use for other users of ID13. We would like to express our thanks to our local contact Manfred Burghammer and the beamline staff of ID13 for the great support provided during the experiment.