ESRF	**Experiment title:** Morphogenesis of highly ordered mesoporous structures produced by single cell organisms	**Experiment number:** LS-3034
Beamline: ID16a	**Date of experiment:** from: 02 Sep 2021 to: 07 Sep 2021	**Date of report:**
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Background:

During the last few decades, significant progress has been made in understanding key biochemical mechanisms responsible for biogenic mineral formation. However, little is known about the physical forces and thermodynamic constraints that regulate the morphogenesis of biomineralized tissue on the macroscopic scale. Our recent work shows that the formation of mineralized ultrastructures in a variety of calcium carbonate- and amorphous silica-based tissues is the result of a thermodynamically driven self-assembly processes.[1–3] The control over the morphology of mineralized tissue was shown to be exerted by the organisms by setting the necessary physical and chemical boundary conditions in which the different mineral architectures grow spontaneously. Nevertheless, physical driving forces that govern the morphogenesis of mineralized walls of single-cell algae, such as diatoms and dinoflagellates, remain a mystery. A number of studies suggest that structural evolution of these complex and highly ordered porous architectures is the result of cellular liquid-liquid phase separation processes in which the deposition of the inorganic material is templated by one of the formed organic domains. However, this assertion was never validated as, so far, the process of algae cell wall biomineralization in time and in space was never described. In this work, we followed the formation of mesoporous mineralized walls in single cell organisms, namely, diatoms from *Coscinodiscus sp.*[4] and dinoflagellates *L. granifera*[5]. This was achieved by tomographic imaging of the cells at different stages of their formation in cryogenic conditions. These data are crucial for numerical simulations of phase separation and solidification processes in these cell walls using phase-field and other approaches performed in our group. Moreover, as the main function of these tissues is mechanical, we intend to investigate the mechanical efficiency of these mesoporous structures, which required a detailed structural information on the studied species in 3D.

Experiments and Setup at ID13

To study the nano-architectures mentioned above, we used the holotomographic imaging method on the beamline ID16A-NI. The exceptionally small beam and the resulting nanometer-sized resolution matches well the approx. 100 nm feature size that we needed to resolve. We followed the morphological evolution of the two investigated organisms at five consequent stages of their formation. The imaging beamline ID16A-NI made it possible to perform zoom-projected imaging that was combined into phase-retrieved holotomographic reconstructions of the small samples. We investigated a total of 10 samples containing a number of frozen cells

collected at 5 different stages of their development – five samples per organism. Furthermore, we performed both „low" resolution holotomographic overview scans and also local-tomography using the higher resolutions of the experimental setup, of 40 nm. The holotomography datasets were phase retrieved to quantify local structural variations in density and reconstructed to create 3D models of the cells at different stages of growth.

Analysis and Results

The high flux, high resolution, cryogenic setup, dedicated software and staff experience on ID16A provided us with unprecedented 3D reconstructions leading to new insights into the growth process and performance of these single cell architectures. An example of a 3D reconstruction of two dinoflagellates and a single dividing diatom cell are presented in Figure 1A and 1B, respectively.

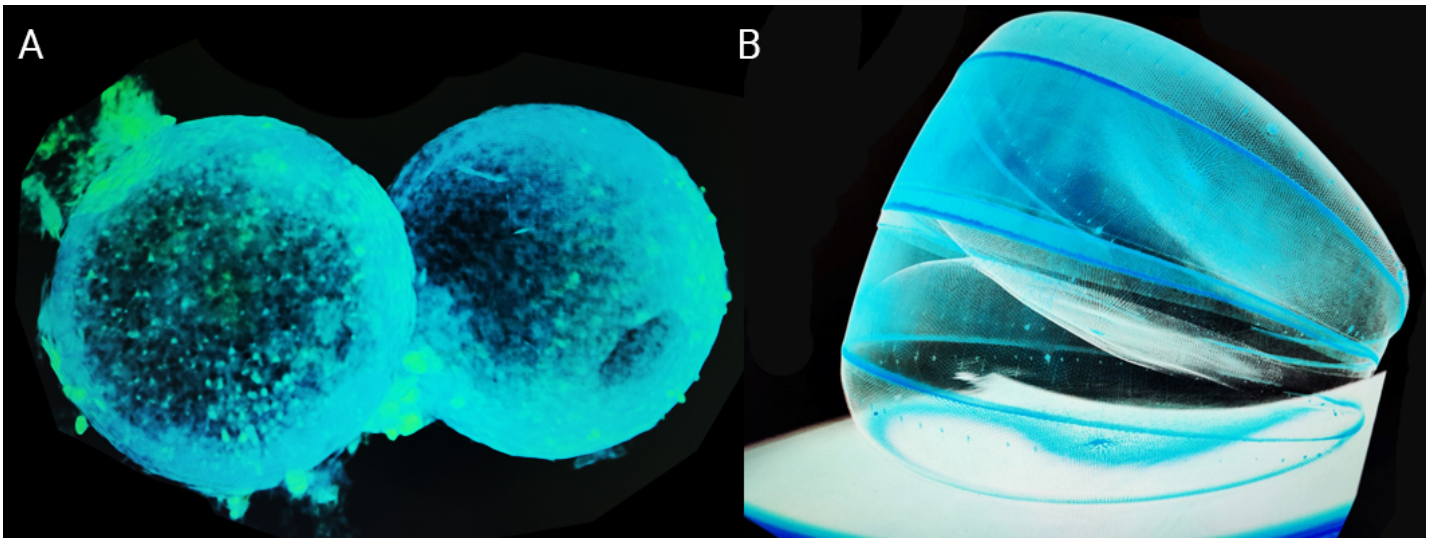

Figure 1: 3D reconstruction of (A) two dinoflagellates *Leonella granifera*, cell diameter is 15 micron; and (B) a dividing diatom from *Coscinodiscus sp.*, cell diameter is 120 micron.

Currently these data are being processed and segmented as part of a master thesis with the goal to follow the development of the mineralized cell wall during its formation.

Impact

The study provided unprecedented information on mineral morphogenesis during these unique biomineralization schemes in single cell algae species. Currently, we are developing a computational model that describes the formation of these complex and highly ordered porous structures. The model is based on the data obtained during the experiment on ID16A. Finally, a master thesis will be written based on this work. The manuscript is currently in preparation.

Bibliography

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