ESRF	**Experiment title:** **Coherent Diffraction Imaging of coccoliths: a new way for measuring the mass for a range of ecologically and biogeochemically relevant coccolithophores**	**Experiment number**: **EV-162**
Beamline: ID10	**Date of experiment**: from: 2nd Nov. to: 9 Nov	**Date of report**: 10.10.15
Shifts: 15	**Local contact(s)**: F. Zontone	*Received at ESRF:*

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Report:

Coccolithophores are abundant unicellular phytoplankton that secrete calcium carbonate plates (called coccoliths) consisting of a radial array of elaborately shaped calcite crystal units. Coccoliths surround the cell, forming a composite exoskeleton called a coccosphere (Fig. 1). Coccoliths are produced in the sunlit surface layer of the ocean and some sink to the ocean interior upon cell death, meaning that coccolithophores play a key role in the global carbon cycle. Oceans sequester 20-35% of CO_2 emissions related to human activities, resulting in the progressive lowering of ocean pH which is thought to be likely to significantly impact biogenic calcite precipitation (Orr et al. 2005). In culture experiments, coccolith calcification was initially reported to decrease upon increasing ocean acidification (Riebesell et al., 2000), but subsequent studies have shown inter-specific and even intra-specific variability in the response to acidification (e.g. Iglesias-Rodriguez et al. 2008). The mass of most modern coccoliths is around 10 pg (10×10^{-12}g) for a size of few micrometers and it is thus impossible to directly weigh them. Coccolithophore specialists currently use an indirect method for estimating their mass using the birefringence of the calcite mineral in cross-polarized light microscopy (Beaufort, 2005; Beaufort et al., 2014). This method is increasingly used on modern-day and fossil coccolith samples (e.g. Beaufort et al. 2011), but would significantly benefit from fine-scale calibration by precise measurements of the structure, volume and mass of coccoliths by Coherent Diffraction Imaging (CDI). For this, CDI is a highly pertinent technique to accurately probe coccolith and coccosphere structure provided that one can deposit these objects onto Si_3N_4 membranes with a sufficient precision so as to retrieve for CDI analysis.

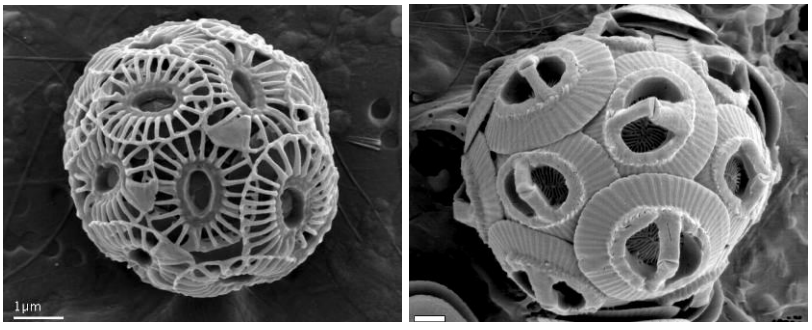

Figure 1: (a) Scanning Electron Microscopy images of coccospheres of two species of coccolithophore: *Emiliania huxleyi* (left) and *Gephyrocapsa oceanica* (right).

Results:

In this run we have tried to image a series of coccospheres by CDI. The run started quite poorly as although we did prepare the membranes beforehand it was exteremly difficult to (1) locate the right particle to be imaged on the membrane and (2) to be certain that the size of the object would be adapted to the resolution dictated by the distance between the sample and the detector. We basically lost half of the run to properly handle this problem until B. Suchéras-Marx suggested to use the enviromental SEM loacted at ID21 to pinpoint the right particle to be imaged and its location on the membrane. This was the beginning of very succesful CDI acquisitions that allowed us to demonstrate the feasibility of the expected objectives of this experiments.
A show in Fig. 2 we were able to first image the entire coccosphere and to retrieve thanks to the courtesy of Paul Tafforeau and Vincent Fernandez at ID19 to extract some of the coccoliths from the coccosphere by using the GVStudio software available at their beam station.

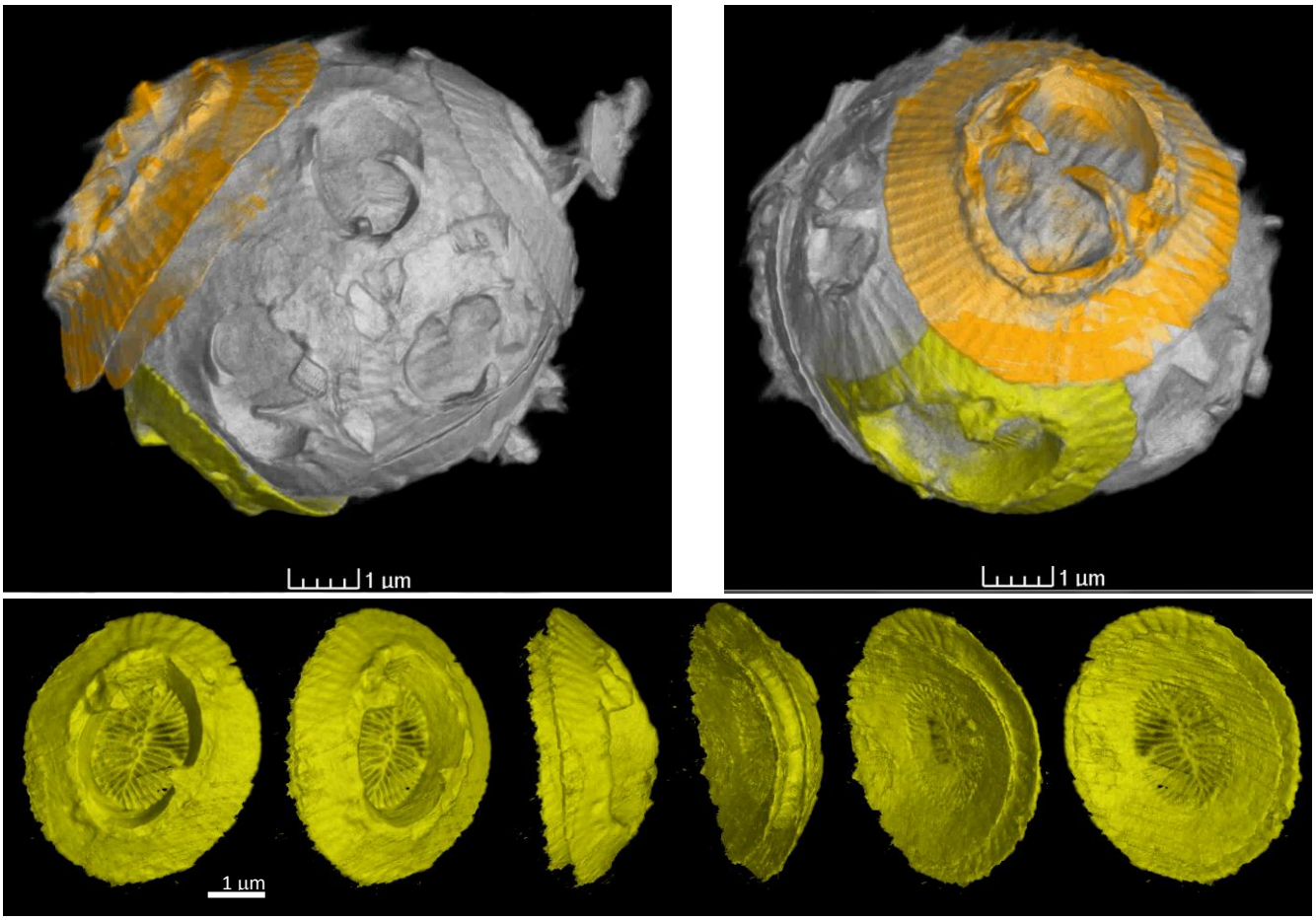

Figure 2 : CDI Reconstruction of a coccopshere of *Gephyrocapsa oceanica* together with the extraction of two segments of the image showing the coccoliths (yellow and orange colors). On the bottom pannel the view of the coccoliths after extraction from the coccospheres at 6 different viewing angles.

As shown in Fig.2 , the 3D array of the coccolith extracted from the image provides quantitative information about the volume, the size and the mass of a single coccolith this meeting the requirements of the objectives of this experiment! To the best of our knowledge it is the first time that a full image in 3D of a coccosphere containing all these details was achieved. The data analysis is now in progress.

As mentionned in the report the main difficulty we are facing now is the lack of a complete dataset spanning several species to allow a statistical estimation of the mass of coccoliths in order to fully validate our objetives. We consider that we will need another run to achieve this goal. The team is now fully trained for this kind of experiment both in terms of experimental requirements and of data analysis.

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