EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:**	**Experiment number**: ES841
Beamline: ID16B	**Date of experiment**: from: 31/10/2018 to: 4/11/2018	**Date of report**:
Shifts: 12	**Local contact(s)**: Vanessa SUAREZ	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Cinzia Bottini, Università di Milano, Dipartimento di Scienze della Terra*

Monica Dapiaggi, Università di Milano, Dipartimento di Scienze della Terra*

Nicola Rotiroti, Università di Milano, Dipartimento di Scienze della Terra*

Report:

The experiment was about the XRF analysis, in very high resolution, of isolated coccoliths of minute dimensions (from 2 to 10 μm, depending on the species). Some of the coccoliths were actual, coming from cultures performed by our working group (Faucher et al., 2017), other were taken from geological samples of early Cretaceous age. Coccoliths are produced by phytoplanktonic algae called coccolithophores and are made of calcite. The main objective of our analyses was to map the concentration and distribution of trace elements (Sr and lighter elements) in coccoliths cultured in normal VS stress conditions (high CO_2 and metal concentrations) as well as in fossil coccoliths from geological intervals favorable for calcification VS intervals of extreme paleoenvironmental conditions.

The experiment was very successful as we managed to analyze a relatively large number of samples with a resolution of 50 x 50 nm, and a few larger ones with a resolution of 100 x 100 nm for a total of 20 specimens analyzed. This allowed to compare the results between specimens collected from the same culture conditions thus providing a more solid interpretation of the outcomes. The results obtained are stunning.

Fig. 1 shows a high-resolution image at 50 nm resolution of a *Coccolithus pelagicus* (extant species of about 8 μm). The red colour represents Ca concentration, while the yellow one represents Sr concentration. The figure shows some areas where both elements are present (though in different concentration) and areas where only one of two is present. Sr can enter calcite structure, so it's very likely distributed almost everywhere in the sample. Other elements, such as Cl, for instance, are present in the culture (as the coccolithophores which produced the coccoliths to form their shell, live in seawater), but do not usually enter the calcite structure.
Fig. 2 shows the concentration distribution of Cl and Ca in another *C. pelagicus* specimen. As it can be clearly seen in the figure, Cl is distributed over the whole sample, but it's more concentrated in certain areas, where Ca decreases.

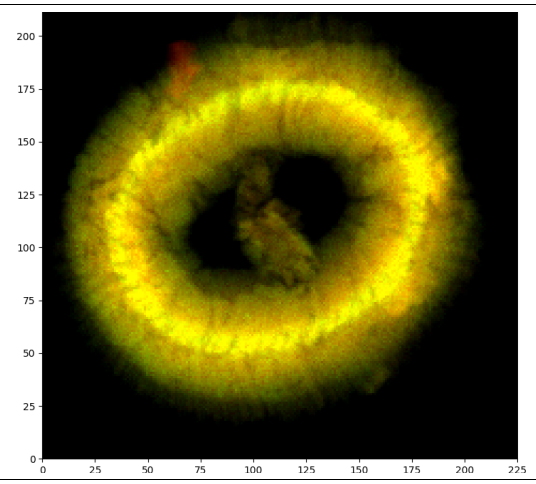

Fig. 1 – Specimen of *Coccolithus pelagicus* (sample C9-cp7). In red Ca concentration, in yellow Sr concentration, with a resolution of 50 nm

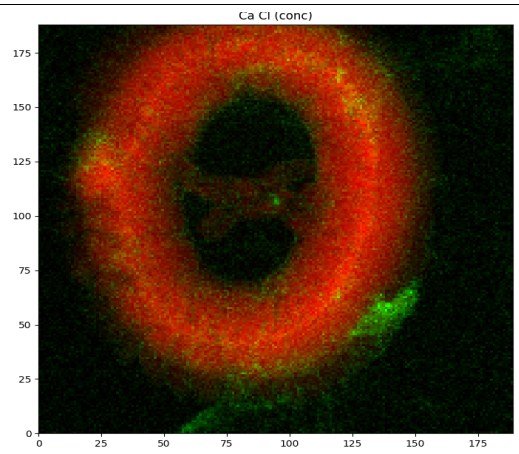

Fig. 2 Specimen of *Coccolithus pelagicus* (sample C9-cp4). In red Ca concentration, in green Cl concentration, with a resolution of 50 nm.

The experiment was challenging, particularly for what concerns the sample preparation. Such small samples are difficult to isolate, extremely difficult to handle and to deposit on the sample grid. We used a TEM sample holder, with a Cu grid with grid elements. This was a winning choice for what refers to finding the specimens which were easily detected with the usual methods suggested by the beamline scientists. However, the presence of copper was inadequate, as its signal tended to mask the signal coming from the sample. For the next experiment, we plan to use a different sample holder.

The results of experiment ES841have been submitted to Scientific Reports (Bottini C., Dapiaggi M., Erba E., Faucher G., Rotiroti N. "*High resolution spatial analyses of trace elements in coccoliths reveal new insights into element incorporation in coccolithophore calcite*" under review, SREP-19-41998-T), the abstract of the paper is as follows:

<< Coccolithophores are phytoplanktonic algae which produce an exoskeleton made of single platelets of calcite named coccoliths. They are widespread in all oceans and directly impact the long-term C cycle. The study of coccolith size, morphology and composition reveals important information regarding the ability of the cell to calcify and on the factors that alter this process. In this regard, very little is known about coccolith elemental composition and if this changes under altered environmental conditions. Here, we present high resolution (50x50 nm) elemental spatial distribution in pristine coccoliths of *Coccolithus pelagicus* and *Gephyrocapsa oceanica* reconstructed via X-ray fluorescence analyses at synchrotron. The studied specimens are from control culture and metal-enriched (V, Ni, Zn and Pb) experiments. The specimens produced under stress conditions display an irregular shape and are thinner, especially in the margin, being marked by a reduction of 1/3 in the total amount of Ca. The same specimens also have higher Sr/Ca ratio with highest values in the coccolith margin, suggesting that difficulty in calcification is additionally reflected in altered Sr partitioning. Two elements, As and Se, are found in the coccolith calcite suggestive of other cations incorporation in the calcite following the Ca pathways. Coccoliths are not the detoxing reservoirs of the metals added in higher concentrations. V and Pb apparently did not interact with the coccolith but Zn and Ni were deposited on the coccolith surface. >>