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

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

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.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal 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:** Analysis of marine adhesives of diatom algae with nanoprobe X-ray fluorescence	**Experiment number**: EV-182
Beamline: ID16A-NI	**Date of experiment**: from: 11.05.2016 to: 13.05.2016	**Date of report**: 16.11.2016
Shifts: 6	**Local contact(s)**: Yang Yang, Sylvain Bohic	*Received at ESRF:*

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Report:

In the experiment "Analysis of marine adhesives of diatom algae with nanoprobe X-ray fluorescence" we aimed to investigate the composition of the adhesive of the diatom *Navicula perminuta*. The diatom *N. perminuta* is a macrofouler with a length of around 10 µm and prevalent on a range of modern fouling-release coatings. The diatoms were cultivated in our laboratory in Bochum and settled on vacuum compatible Kapton foils (8 µm thickness). After removing the non-settled algae and the medium with Milli-Q water, the samples were air-dried. Subsequently some algae were removed by a water flosser to to analyze the remaining adhesive. All samples were pre-characterized with light microscopy. The regions where algae were settled were marked before removal with the water flosser to be able to quickly access the regions of interest during the beamtime

During the beamtime at ID16A-NI the photon energy was tuned to 17.05 keV to cover the K-emission lines of the metals of interest. We performed 2D XRF scans with a step size of 50 nm and a dwell time of 50 ms. The used focal spot size was 33 x 55 (horizontal x vertical) nm^2.

Due to the very stable beam we were able to effectively use of the whole 6 shifts and measure 15 diatoms with their adhesive. 2 algae and one measurement of the adhesive on hydrophobic surface are shown in Fig. 1.

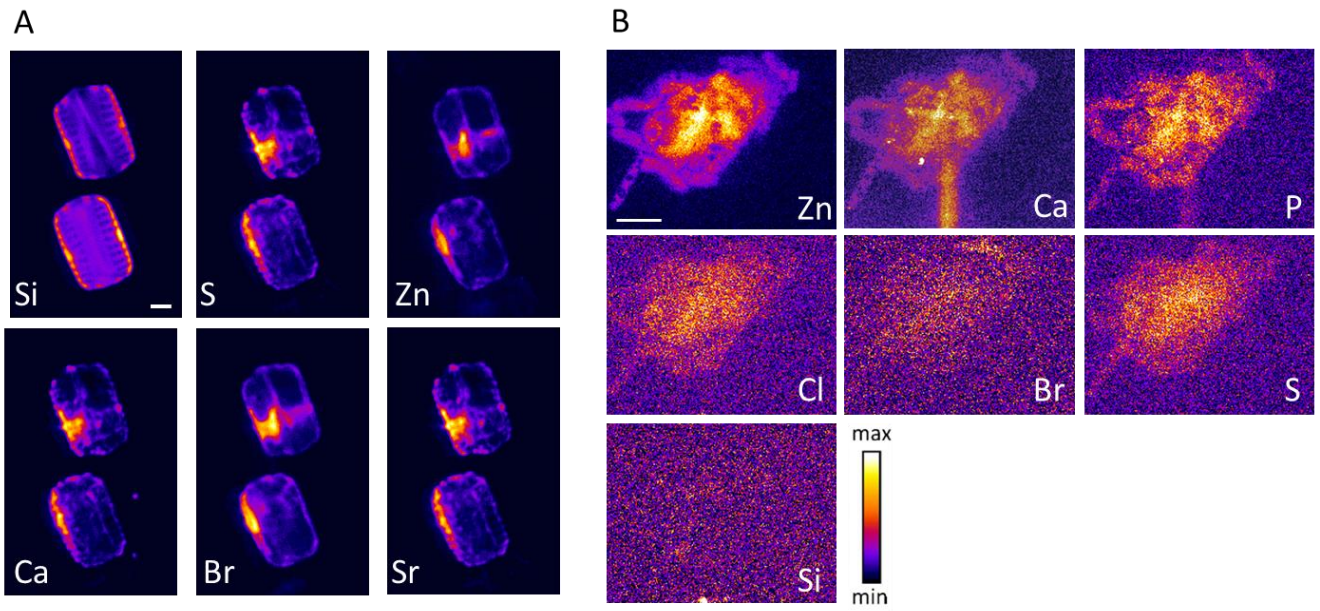

Figure 1: A: Fluorescence signals of the alga shell. The scale bar is 2 µm. B: Fluorescence signals of the adhesive after removal of the alga. The scale bar is 1 µm.

The algae shell which consists mainly of silicon was clearly visible in the XRF images obtained with the nano focused beam. Besides Si the shell consists of calcium, sulfur, zinc, bromine and strontium (see Fig. 1A). The data reveals that some elements like calcium and sulfur as well as bromine and zinc were spatially correlated. At the sample set with the remaining adhesive without any algae we found a high concentration of zinc in the adhesive (see Fig.1B). This zinc signal was correlated with calcium, bromine, chlorine, sulfur and also phosphorus. The missing silicon signal shows that the alga were fully removed without any residues.

As further goal we wanted to test the hypothesis if the composition of the adhesive varied on surfaces with different chemical termination. For that we compared hydrophobic Kapton foils to hydrophilic Kapton foils. While lower number of algae settled on the hydrophilic surface there were also slight differences in the fluorescence signal. While the signals of the algae itself were the same on both surfaces, the adhesive on the hydrophilic surface had slightly more bromine and zinc in it. Another difference was that we found a low silicon signal in the remaining adhesive on hydrophilic surfaces while no silicon was found on hydrophobic surfaces. This could be a hint that there are still residues of the algae on the hydrophilic surface after removal.

In former experiments we found evidence for the presence of Br in the adhesive. To answer the question whether bromine is secreted by the algae or if the extracellular polymeric substance of the diatom absorbs the bromine from the ambient sea water environment we changed the culture medium. The algae were grown in media without addition of bromine before settling on Kapton foils. For algae that attached under these conditions we still found bromine signals in the adhesive which means that the bromine was secreted by the diatom.

Concluding, our experiments revealed that the pure adhesive without residues of algae can be detected and cleary distinguished from not completely detached algae. The established approach to distinguish between secreted and post-adsorbed elements opens up a variety of experiments for future X-ray nanoprobe investigations of marine adhesives.