ESRF	**Experiment title: Unveiling Ca^{2+} requirements for light acclimation in diatoms**	**Experiment number**: LS-3134
Beamline: ID21	**Date of experiment**: from: 27/10/2022 to: 01/11/2022	**Date of report**: 06/06/2023
Shifts: 12	**Local contact(s)**: Luis Carlos Colocho Hurtarte	*Received at ESRF:*

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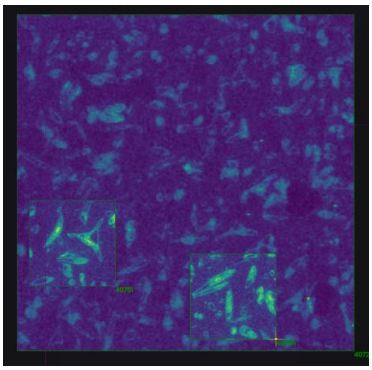
Report:

We performed the XRF imaging experiment in vacuum, at room temperature, with an incoming beam energy of 7.3 keV. The beam was focussed to 0.83×0.23 μm^2.

The samples were the following diatomes:

- *Phaeodactilum Tricornutum*, Pt18 strain, in which four different morphotype coexist (cruciform, triradiate, oval and fusiform), grown in normal light conditions
- *P Tricornutum*, Pt18 strain, grown in "high light" conditions (HL)
- *Thalassiosira oceanica*
- *Thalassiosira weissflogii*
- *Thalassiosira pseudonana*

Cells were cryo-fixed with high pressure freezing, then cryo-substituted and embedded in resin. Ultrathin sections (300 nm) were obtained using an ultra-microtome, laid on Si_3N_4 windows, and then analyzed on ID21. The main goal of the experiment was to measure the elemental distributions in the four different morphotypes of the Pt18 strain, and evaluate how these are affected by the illumination condition.



We adopted a general strategy to explore large areas of the section containing several cells, in which the different morphotypes could be identified:

1. Acquisition of an exploratory XRF map: 98×98 μm^2, 0.5×0.5 μm^2 step, 200 ms/pt dwell time.
2. Acquisition of high-resolution XRF maps in selected areas where the morphotypes of interest were localized: 25×25 μm^2, 0.25×0.25 μm^2 step, 300 ms/pt dwell time.

An exemple of acquisition of exploratory vs high resolution maps is reported in Figure 1.

Figure 1. *Screenshot of the Daiquiri user interface showing two high-resolution 25×25 μm^2 area XRF maps overlaid to a larger XRF map (98×98 μm^2).*

We could acquire high quality data for all samples.
The XRF hyperspectral images were treated with the PyMca software, in order to extract quantitative elemental images. The response of the SDD detector was calibrated over a thin film multilayer sample from AXO.
The distribution of the main elements of interest for this study in the control sample of Pt18 are reported in Figure 2.

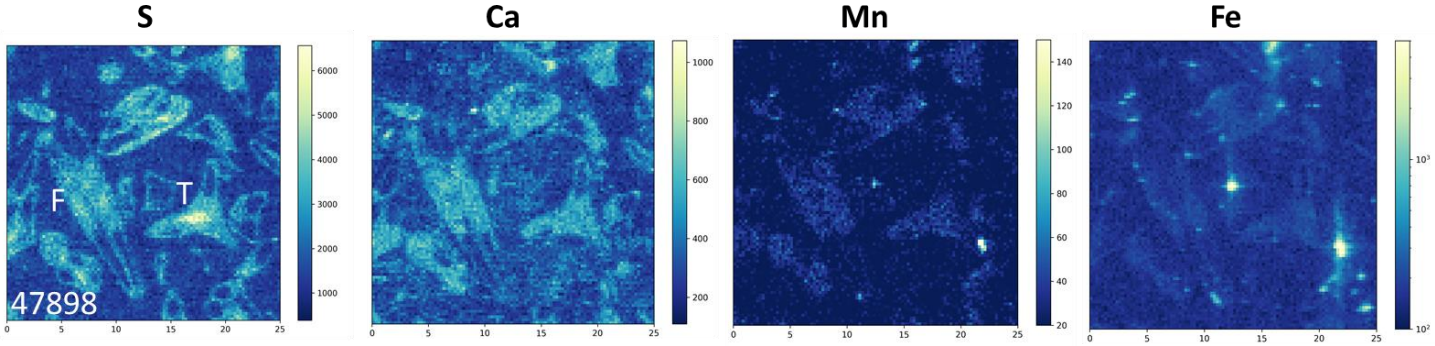


Figure 2. *False-color representation of the concentration of selected elements in a section of resin-embedded Phaeodactilum Tricornutum diatomes. The elemental distributions highlight the presence of different morphotypes: Fusiform (F) and Triradiate (T) are highlighted in the S distribution map. All concetrations are in ppm. The axes labels are in µm. Step size: 0.25×0.25 µm^2.*

This kind of data allowed us to measure the elemental concentrations in the different morphotypes, bringing insight into the natural composition of diatomes and its relation with the polymorphism exhibited by the Pt18 strain. These data are currently included in a publication in preparation, along with electron microscopy and physiological studies.

Additionally, the same experimental protocol was used to explore the elemental content of other diatoms (*T. oceanica, T. weissflogii, T. pseudonana*), revealing interesting and yet unknown co-localizations of elements in specific cellular compartments. An exemple is reported in Figure 3, where the co-localization of Ca (green) and Fe (blue) in the central vacuole of the cell is highlighted. Fe is located also in the cell walls. These data bring insight into the elemental content and subcellular distribution in different ecologically relevant organisms.

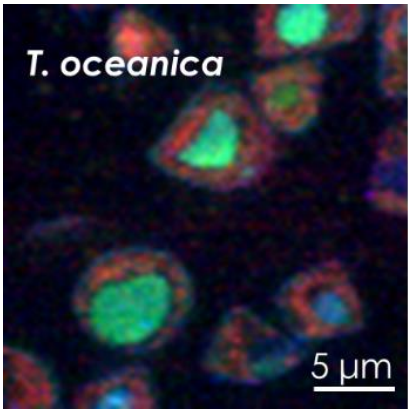


Figure 3. *False-color representation of the distribution of Ca (green), Fe (blue), and S (red) in a section of resin-embedded T. oceanica, exctracted from an XRF hyperspectral image. Step size: 0.25×0.25 µm^2.*

Publication in preparation: Serena Flori et al. “Unveiling diatoms pleomorphic architecture, the case of study of the strain Pt18”