Correlative TOF-SIMS/SEM for subcellular investigation of microalgae in extreme environment

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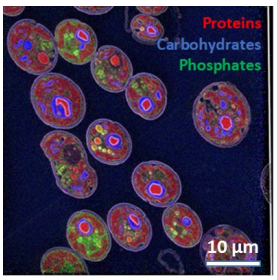
Visualizing the metabolism within its structural context is key to understanding the biological phenomena at stake, especially at the subcellular scale. While biochemical purification may function for specific organelles of model species, this is tedious and often impossible for atypical or less studied organisms. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) enables the combined visualization of elements and molecules and is becoming more and more popular in the field of life sciences, especially for the investigation of tissue samples. Even though the ultimate lateral resolution of TOF-SIMS ought to be sufficient for subcellular imaging, very few studies have yet pushed the lateral resolution that far. In this context, we have investigated how the subcellular architecture and molecular composition of microalgae thriving in extreme environments is affected by the harsh conditions they live in.

For this purpose, we have performed TOF-SIMS imaging, combining both high lateral resolution (up to 100 nm) and high mass resolution (up to 9,000) over resin-embedded specimen. We also performed over the same zone correlative SEM (10 nm) in order to identify subcellular compartments and used this image to segment the hyperspectral TOF-SIMS dataset. We are thus combining the strength of both techniques and increasing the resolution of TOF-SIMS imaging using SEM-derived information.

We investigated the snow alga Sanguina nivaloides that blooms over lasting snow in the spring season and was recently been identified in the Alps, as well as the uranium-tolerant Coelastrella sp. PCV that has been shown to tolerate extremely high doses of uranium. In these algae, we have been able to perform molecular profiling of several subcellular features, including cell walls as thin as ~ 100 nm, nuclei, pyrenoid, starch granules and platelets and chloroplasts. In the case of S. nivaloides especially, the environmental samples hint at a likely interaction between the alga, mineral particles and bacteria that could explain how the alga is able to collect micronutrients in its hostile snow environment. Coelastrella on the other hand is able to remodel its compartments and their metabolic content when submitted to uranium-related stress.

Our TOF-SIMS images almost meet NanoSIMS images (that is however restricted to purely elemental mass spectrometry imaging) in term of resolution. Correlative TOF-SIMS/SEM allows a remarkable enhancement of the comprehensibility of TOF-SIMS images by adding in extra structural information. We reveal for every different organelle a different metabolic footprint and hints into their adaption to their specific environment.

Graphic:



Keywords:

Metabolic imaging, Microalgae, Correlative microscopy

Reference:

Ezzedine, Jade A., et al. "Adaptive traits of cysts of the snow alga Sanguina nivaloides unveiled by 3D subcellular imaging." Nature Communications 14.1 (2023): 7500.
Beaulier, Camille, et al. "Characterization of a uranium-tolerant green microalga of the genus Coelastrella with high potential for the remediation of metal-polluted waters." Science of The Total Environment 908 (2024): 168195.