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NanoSIMS ion imaging analyses for biological samples: Applications to sebseafloor life.

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The CAMECA NanoSIMS 50L ion microprobe represents the *in-situ* microanalysis by secondary ion mass spectrometry, combining unprecedented spatial resolution (minimum spot size of ˜50 nm for Cs^+ or ˜150 nm for O^-) with ultra-high sensitivity. Up to 7 elemental and/or isotopic images can be acquired simultaneously by 7 electron multipliers with sensitivity in the ppm. The capability for maps of multiple elements and isotopes within a sample with permil precision and accuracy and nm scale spatial resolution is unique to the NanoSIMS and provides a new approach to study of the isotope and trace element distributions within the sample, i.e., extraterrestrial, terrestrial and biology samples, including meteorites, Earth rocks and microbial cells in deep and ancient subseafloor sediments by the Integrated Ocean Drilling Program (IODP).

In last decade conventional SIMS technique has been used to microbiology to match chemotaxonomic and phylogenetic signature of microbes. Recently NanoSIMS ion imaging introduced to a stable isotope probing study (i.e., ^{13}C, ^{15}N labeling) for a single cell to understand microbial metabolic activities, and metal-probed *in-situ* hybridization for phylogenetic identification.

Subseafloor sediments of the South Pacific Gyre (SPG) obtained by IODP Expedition 329 represent a large proportion of organic-poor, oxidized sediments in the open sea. The sediment is characterized as rich in oxygen but poor in energy sources. In an energy-limited sedimentary environment, a small size of microbial community perseveres functions for life with extraordinary low oxygen-consumption rate. However, the nature of deep sedimentary microbial life in the SPG remains still unknown. In this study, we will investigate metabolic activity of the SPG sedimentary cells with a NanoSIMS ion imaging.

Isotope labeled SPG sedimentary cells (incorporation of substrates after 1.5- years incubation) were analyzed by a raster ion imaging in a NanoSIMS 50L ion microprobe at the JAMSTEC Kochi Institute for Core Sample Research. A focused primary Cs^+ beam of ˜0.8 pA was rastered over 20 x 20 to 28 x 28 micrometer areas on samples. Negative secondary ions of ^{12}C, ^{13}C, $^{12}C^{14}N$, $^{12}C^{15}N$ and ^{32}S were measured using 5 electron multipliers in multidetection mode at a high mass resolution of about 9,000 that is sufficient to separate all relevant isobaric interferences (i.e., ^{13}C on $^{12}C^{1}H$). Each run was started after stabilization of the secondary ion beam intensity following presputtering of approximately 5 to 10 min with strong primary ion beam current. Each image run repeatedly scanned (30 to 40 times) the same area, with individual images consisting of 256 x 256 or 512 x 512 pixels, depending on the region-of-interest, having a dwell time of 2,000 to 3,000 microsecond. We prepared E.coli cells as the standard samples, which have different carbon isotopic rations of 0, 5, 10, 15 and 20 % enriched in ^{13}C (relative to the ^{12}C) or ^{15}N (elative to the ^{14}N) to evaluate an instrumental mass fractionation for C and N isotopes as well as to find target mass peaks (^{12}C, ^{13}C, $^{12}C^{14}N$, $^{12}C^{15}N$ and ^{32}S). This presentation will highlight results to illustrate critical analytical issues affecting precision and accuracy including sample preparation and data processing.

Keywords: NanoSIMS, sebseafloor microbes, isotope imaging, stable isotope labeling