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Simultaneous imaging of microbial identity and function by NanoSIMS

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Microorganisms are key players driving the biogeochemical cycling of elements on our planet. Understanding the role of microbes and their involvement in a certain biogeochemical process as active members of microbial communities by establishing the link between microbial identity and metabolic activity remains a major gap in our ability to explore the microbial world. Recent technological advancements in mass spectrometry have created new research opportunities in biogeosciences by the development of imaging mass spectrometry. Such an example is nanometer-scale secondary ion mass spectrometry (nanoSIMS) which can provide high lateral resolution and sensitivity at micron and sub-micron level and was successfully used to image metabolically active microbial cells. Here we describe a novel method that combines nanoSIMS, stable isotope labeling and halogen *in situ* hybridization (HISH) for simultaneous imaging of identity and function of single microbial cells in the environment. Our results show the huge potential of this combined approach for both molecular ecologists and biogeochemists by providing access to the ecophysiology of microorganisms *in situ*, as well as for traditional microbiologists, by offering insight into the physiology of microorganisms obtained in cultures and enrichments. We show examples of studies where ^{15}N and ^{13}C isotope labeling experiments were conducted, followed by halogen *in situ* hybridization and nanoSIMS analysis to identify microorganisms responsible for anaerobic hydrocarbon degradation, nitrogen and carbon assimilation, and to quantify uptake rates and nutrient fluxes in complex microbial communities.