Advanced geobiology answers with a CAMECA NanoSIMS at Caltech

A SUCCESS STORY

PHOTO: V. ORPHAN

Research team (left to right): Ally Pasulka, Meghan Newcombe, and Yunbin Guan

CAMECA NanoSIMS 50L Advantages

- Unique high-performance ion microprobe/secondary ion mass spectrometer (SIMS)
- Utmost-precision isotopic & trace element analysis

- High spatial resolution (down to 50 nanometers)
- High sensitivity (ppm in element imaging)
- High mass resolution (M/dM)
- Parallel acquisition of seven masses

- Environmental microbiology
- Cell biology
- Geology and space science
- Materials research

The Researcher

The highest levels of scientific achievement are often distinguished by a kind of symbiotic relationship between hard work and bright ideas. That link is exemplified by the career of Dr. Victoria J. Orphan.

A relatively young leader in the comparatively young discipline of microbial geobiology, she's already achieved the position of James Irvine Professor of Environmental Science and Geobiology, as well as becoming Alan V. C. Davis and Lenabelle Davis Leadership Chair, Center for Environmental Microbial Interactions (CEMI) — all in the Division of Geological and Planetary Sciences (GPS) at Caltech in Pasadena, California. And among other honors and awards, she was the 2016 recipient of a MacArthur Fellowship (commonly called the "genius grant.")

The Work

The research of Dr. Orphan's hard-working team in her Orphan Lab at Caltech ranges from isotope fingerprinting of microorganisms to peptidoglycan synthesis in insect bacteria to exciting new analyses of individual marine viruses collected from the environment. Much of it is based on a fusion that Dr. Orphan helped pioneer as a graduate student at UCSB with Ed DeLong in collaboration with Chris House at Penn State University and Kevin McKeegan (UCLA): the combination of *fluorescence in situ hybridization (FISH)* to identify individual cells, and ultra-fine analysis using *secondary ion mass spectrometry (SIMS)* to characterize each cell's chemical makeup and isotopic distribution.

For example, ocean-floor emissions of the greenhouse gas methane have gained increasing importance in modeling global climate change. One key study by Orphan's team introduced the use of spatial statistical techniques to examine clusters of symbiotic microorganisms in ocean-floor methane seeps.

The targets: consortia of methane-oxidizing archaea and sulphate-reducing bacteria that help decrease methane release into the environment. The Caltech researchers wanted to analyze their anabolic activity patterns to understand what type of energetic substrate or intermediate was passing between them.

This demanded an analytical instrument with ultra-high resolution — plus innovative thinking from the researchers. "We ended up reconfiguring the way we process our environmental samples," says Dr. Orphan. "We embedded the symbiotic aggregates and cut them very thin so we could maximize the spatial resolution. We needed to resolve individual cells of the symbiotic partnership and compare their anabolic activity with the fluorescence image showing the spatial position of the archaea and bacteria on a one-to-one basis."

The Instruments

After conducting FISH phylogenetic identifications and target mapping in the lab, the team brought the samples to the Caltech Microanalysis Center to run on an advanced CAMECA NanoSIMS 50L. This unique ion microprobe optimizes SIMS analysis performance at high lateral resolution and sensitivity. So it provides superior isotopic and trace element analysis in small regions of interest, with isotope ratio reproducibility of a few tenths of permil. Users report the NanoSIMS 50L delivers key performance metrics simultaneously that other instruments or techniques can only obtain individually.

"The NanoSIMS's spatial resolution is a huge advantage," Dr. Orphan says. "It gives you a clear image of isotopic distribution. And we've worked to boost that resolution, in the analysis of microbial cells and of their ultra-structure. Also, being able to measure seven masses in parallel is really a powerful capability. It allows us to simultaneously track up to three independent isotopes incorporated in the cell. You get a different picture of its overall metabolism than looking at a single isotope at a time.

"Environmental microbiologists, I think, have become quite excited about what NanoSIMS can offer to our field."

Caltech scientists in the GPS Division perform other critical analyses on the center's CAMECA IMS 7f-GEO, a compact, "workhorse" monocollection SIMS model that's ideal for fast, high-precision analyses of trace elements and stable isotopes. For instance, the Orphan team relies on it to deliver averaged isotope values of elements such as sulfur.

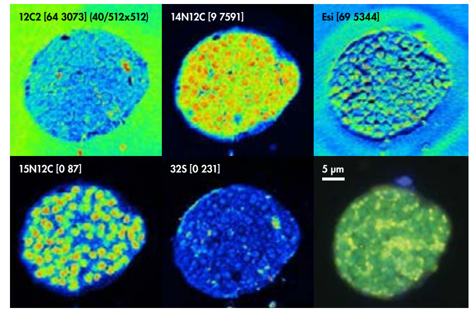
The Results

In the consortia study above, applying the NanoSIMS 50L at full resolution was critical to resolving the puzzle.

"If something was diffusing between the two microorganisms," Dr. Orphan explains, "you would expect cells of the two different symbiont types that were close together would have the highest activity. Cells much further away from the inter-species boundary should have less activity.

"But that's not what we ended up seeing. We created maps of cellular activity and applied spatial statistics, and found that these activity patterns are not consistent with a diffusible substrate. This led to a new hypothesis: interspecies extracellular electron transfer. We found genomic and microscopy evidence that suggests they are creating a conductive matrix containing multi-heme cytochrome proteins — and passing electrons between each other!

"I'm personally very proud of this study," says Dr. Orphan. "It represents a decade of chipping away at the mystery of the methane-oxidizing symbiosis, and coming up with clever ways to analyze this uncultured environmental microbial partnership — using the resolving power of the instrument and this single-cell isotope technique — to get at an answer."



Fluorescence in situ hybridization combined with nanoscale secondary ion mass spectrometry (FISH-nanoSIMS) analysis.

About the Institution

The Division of Geological and Planetary Sciences (GPS) is one of six academic divisions at the California Institute of Technology (Caltech), a world-renowned science and engineering institute in Pasadena, California, USA. The Division's Caltech Microanalysis Center (CMC) provides hardware and expertise for microanalysis of geological, meteoritic, and synthetic materials. CMC's laboratory houses advanced instruments for in situ chemical and isotopic analysis of solids — encouraging innovation in microanalytical techniques and interdisciplinary research among geochemistry, planetary science, biology, and material science and engineering.

About CAMECA

CAMECA is a world-leading supplier of microanalytical and metrology instrumentation for research and process control. Our instruments measure elemental and isotopic composition in materials down to atomic resolution. Advanced CAMECA technologies include secondary ion mass spectrometry (SIMS), atom probe tomography (APT), electron probe microanalysis (EPMA), and low-energy electron-induced X-ray emission spectrometry (LEXES). We address challenging characterization needs in diverse markets, from life sciences, geology, materials sciences, and cosmochemistry to environmental, nuclear, and semiconductor research.

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