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SCIENCE & METROLOGY SOLUTIONS

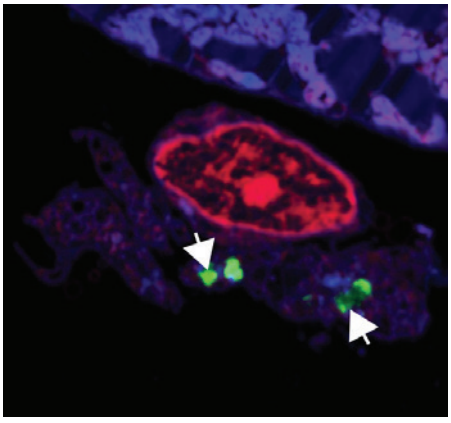
AMETEK
MATERIALS ANALYSIS DIVISION

Probing novel therapeutics possibilities with a CAMECA NanoSIMS at UWA

A SUCCESS STORY

CAMECA NanoSIMS 50L Advantages

- Unique high-performance ion microprobe/secondary ion mass spectrometer (SIMS)
- Utmost-precision trace element & isotopic analysis
- High spatial resolution (down to 50 nm)
- High sensitivity (ppm in element imaging)
- High mass resolution (> 10 000 M/dM)
- Parallel acquisition of seven masses
- Cell biology
- Geology and space science
- Environmental microbiology
- Materials research



Note high levels of Br enrichment in endolysosomes (see arrows) of heart microphages; could ASO therapeutics help treat cardiac allograft rejection?



The labs at UWA pack superlative analytical power, including three CAMECA instruments. Above: the advanced NanoSIMS 50L ion microprobe.

The Lab

Good scientists don't take "no" for an answer. They take it for a good start. That's especially true at a place like the University of Western Australia (UWA) in Perth, which has assembled a powerful array of analytical solutions at its Centre for Microscopy, Characterisation, and Analysis (CMCA). Studies at CMCA's Microscopy Australia Flagship Ion Probe Facility rely on instrumental support by Dr. Paul Guagliardo, a senior research officer and NanoSIMS specialist, along with research fellow Dr. Jeremy Bougoure.

The CMCA lab team persistently pursues answers to questions facing researchers in geology and mining, environmental biology, medicine, molecular biology, material science, and more. "Lately I did the instrument work for a study on how heat stress contributes to dangerous coral bleaching," says Dr. Guagliardo. "But there's a different microanalytical project every week or two. We get researchers from all over the country, and from outside Australia."

The Challenge

One recent UWA study in collaboration with UCLA and Ionis Pharmaceuticals focused on a fascinating molecular biology topic: *nucleic acid therapeutics (NATs).*

If nucleic acids such as DNA and RNA are the building blocks of life, NATs have become the building blocks of an emerging class of next-generation pharmaceuticals. Their capabilities for targeting disease at the genetic level, by preventing the expression of disease-causing proteins, have jump-started a race to develop NAT-based drugs that treat cardiovascular illness, diabetes, cancers, and a host of other human maladies.

However, achieving sufficient understanding of cellular uptake and distribution in tissues — critical for precise drug targeting — has faced significant challenges. The sensitivity and resolution of traditional confocal microscopy fall short when trying to study epithelial cells as thin as 70 nanometers (nm). Another approach, attaching large fluorophore molecules as markers, is susceptible to unwanted changes within the cell caused by the targeted molecules themselves.

Their NATs study needed to avoid these and other limitations. So the CMCA team — with Dr. Haibo Jiang, group leader and DECRA research fellow in UWA's School of Molecular Sciences, one of the study's co-authors, also contributing analytical instrument assistance — turned to NanoSIMS.

The Instrument

The UWA facility's NanoSIMS 50L ion microprobe is made by CAMECA — the pioneering manufacturer of advanced secondary ion mass spectrometry (SIMS) technology. (In a unique concentration of analytical power, CMCA also possesses an earlier NanoSIMS 50 plus an IMS 1280 large geometry ion microprobe.) The NanoSIMS 50L enables lateral resolution down to 50 nm, parts per million (ppm) sensitivity in element imaging, mass resolution greater than 10 000 M/dM, and parallel acquisition of 7 masses. So it can provide superior isotopic and trace element analysis in nanoscale regions of interest.

"What's special about NanoSIMS is the high mass resolution and high spatial resolution together with high sensitivity," says Dr. Guagliardo. "That combination allows you to answer a lot of questions."

The Work

The NATs project concentrated on modified *antisense oligonucleotide (ASO)* NATs. In a key portion of the study, researchers labeled ASO solutions with bromine (otherwise present in tissues only at much lower levels), then injected them into wild-type mice. (Another study branch proposed using NanoSIMS' unique capabilities for visualizing isotopic labels, by injecting cells with a stable, relatively rare sulfur isotope; but this was not pursued when background cellular isotope levels proved too high.)

Heart, liver, and kidney cells were then harvested, prepared, and imaged.

NanoSIMS is often utilized with complementary techniques. Here backscattered electron (BSE) imaging was also performed, using a scanning electron microscope. NanoSIMS imaging provided nanoscale chemical information (elemental and isotopic content of cells and subcellular structures) while BSE images offered morphological detail with resolution of a few nanometers.

The Results

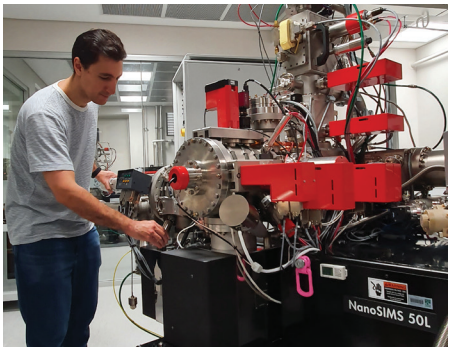
The researchers found that NanoSIMS revealed, with both high sensitivity and high spatial resolution, the distribution of bromine-labeled ASOs throughout cellular and subcellular compartments.

As their paper reports, "NanoSIMS imaging, particularly when combined with correlative BSE imaging, provides novel insights into ASO distribution in cultured cells and mouse tissues. We showed that phosphorothioate ASOs associate with filopodia and the inner nuclear membrane of cells, and we documented substantial cellular and subcellular heterogeneity in ASO distribution in the liver, heart, and kidney."

They concluded that NanoSIMS imaging will be useful in optimizing the efficacy and delivery of NATs for treating human disease.

For molecular biology as well as geoscience, material science, and more, Paul Guagliardo emphasizes that the NanoSIMS is a highly specialized scientific device, which requires trained operation and precise sample preparation, plus carefully chosen and designed experiments. But he believes researchers will find it provides solutions that are otherwise unobtainable.

"If we think that NanoSIMS can solve their problem," he says, "then we certainly recommend it. You've just got to convince people to give it a go."



Dr. Paul Guagliardo preps the NanoSIMS 50L for another run.

Haibo Jiang agrees: "It's been very important for my research. I recommend people to try NanoSIMS if they have the right question."

About UWA

The Microscopy Australia Flagship Ion Probe Facility is located within the Centre for Microscopy, Characterisation, and Analysis (CMCA) at the University of Western Australia (UWA) in Perth. The CMCA enables research excellence by providing world-class microscopy and microanalysis facilities and expertise to publicly funded researchers and industry. Its expert academic, technical, and professional staff support the full user pathway — from initial project discussion and planning, through to application of cutting-edge characterization and analytical techniques, assistance with data interpretation, and publication.

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), and electron probe microanalysis (EPMA). 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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