CAMECA
SCIENCE & METROLOGY SOLUTIONS

NanoSIMS

Cell biology

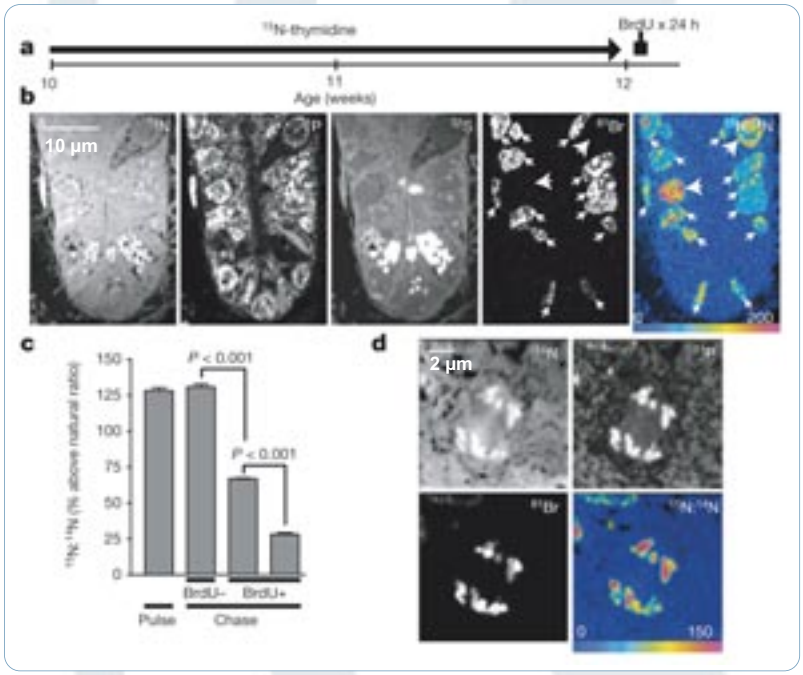
Molecule and trace element imaging at sub-cellular resolution from micro-organisms to human cells

2D & 3D imaging with 50nm resolution

Detection & distribution of stable isotopes and trace elements tagged molecules

Measurements of kinetics & fluxes





Test of the immortal strand hypothesis for stem cell division in the small intestine of mice

a) Pulse-chase experiments: ^{15}N-labelled thymidine and BrdU, administered in vivo to the animal.

b) Elemental mappings (N, P, S) provide histological information. BrdU (Br image) & Thymidine (^{15}N/^{14}N image) highlight new cell production, division and fate, as these markers are specifically incorporated in new DNA.

c) Number of cell divisions derived from quantitative measurement of ^{15}N-thymidine label dilution.

d) Symmetric chromosomal segregation during mitosis phase.

From: Multi-isotope imaging mass spectrometry quantifies stem cell division and metabolism. M. L. Steinhauser et al. NATURE, Vol 481, 26 Jan 2012.

AMETEK
MATERIALS ANALYSIS DIVISION



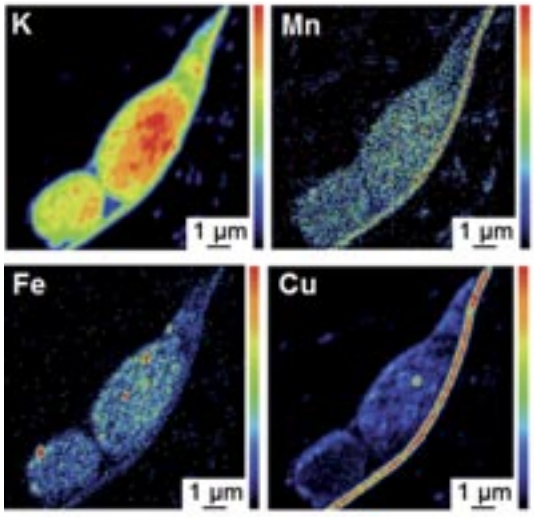
Introduction to the SIMS technique

Secondary Ion Mass Spectrometry (SIMS) is based upon the sputtering of a few atomic layers from the surface of a sample, induced by a *primary ion* bombardment. Molecules are broken, atoms and atomic clusters are ejected, some of them being spontaneously ionized. These *secondary ions* are a characteristic of the chemical composition of the analyzed area. They are separated according to their mass, and images are derived from variations in the signal intensity over the scanned area.

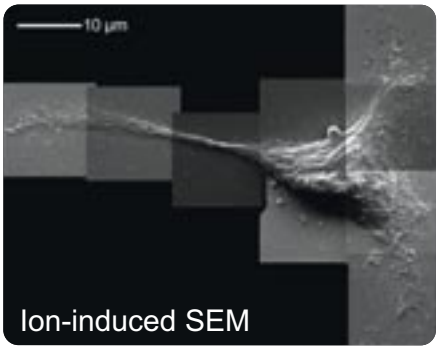
Due to a unique design, the CAMECA NanoSIMS 50L ion micro-probe permits to simultaneously:

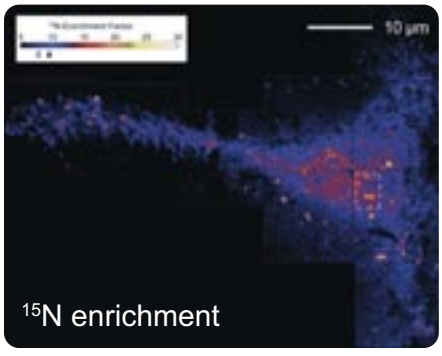
- achieve sub-cellular lateral resolution (50nm),
- work at high mass resolution resolving most interferences (i.e. $^{13}C^{14}N$, $^{12}C^{15}N$, $^{13}C_2H$, all at mass 27 AMU for instance),
- deliver high throughput & sensitivity by working in DC mode (as opposed to pulsed TOF analyzers) and detecting up to seven masses in parallel.

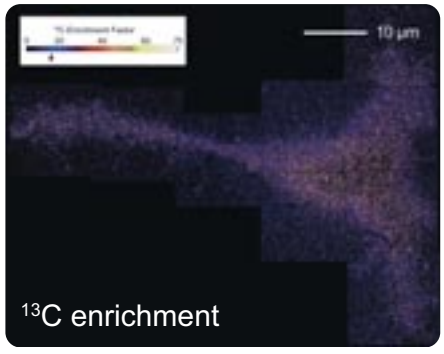
Trace elements can be mapped at high resolution, including light elements (H, Li, Be, B, C, N, O, …) not accessible or having substantial background in analytical TEM or SEM.



Trace element mapping in plants
Arabidopsis thaliana, insight into the chloroplast showing small granules enriched in metals.
Courtesy of D. Schaumlöffel, CNRS, IPREM-Pau, France.







Coupling NanoSIMS with isotopic or elemental labeling

Due to the very low ionization yield of intact molecules, MALDI imaging of molecular ions is limited to a few tens micrometer lateral resolution. Static molecular SIMS is limited to µm level lateral resolution, generally detecting fragmented ions.

The NanoSIMS approach is opposite: using energetic reactive primary ions (O^- and Cs^+), it breaks molecules into more numerous atomic ions, maximizing the atomic ionization yield. The higher sensitivity permits a better lateral resolution.

The different isotopes of one element can be mapped. Dividing one image by another, pixel by pixel, permits obtaining a high resolution isotopic ratio map. It is unique to the NanoSIMS to map distribution of molecules in tissues or cells through the use of tracers, down to 50nm lateral resolution.

One can deliver animals, plants or cells ^{15}N-labelled amino acids, ^{13}C-lipids, a deuterated drug, etc… Not only stable isotopes but also radioactive labels can be used, in continuation of approved protocol (^{3}T, ^{14}C,...). The labels can also be elements that are rare in the environment, i.e. tagging several antibodies in parallel with different rare earth elements; see next page.

Lipids in cell membrane
Mapping the distribution of ^{15}N-enriched ions from metabolically labeled ^{15}N-sphingolipids in the plasma membrane of a Clone 15 fibroblast cell, revealing sphingolipid-enriched plasma membrane domains distinct from lipid rafts. Mosaic of high resolution maps.

From: Direct chemical evidence for sphingolipid domains in the plasma membranes of fibroblasts.
J. F. Frisz et al. PNAS, vol. 110, 19 Feb. 2013.

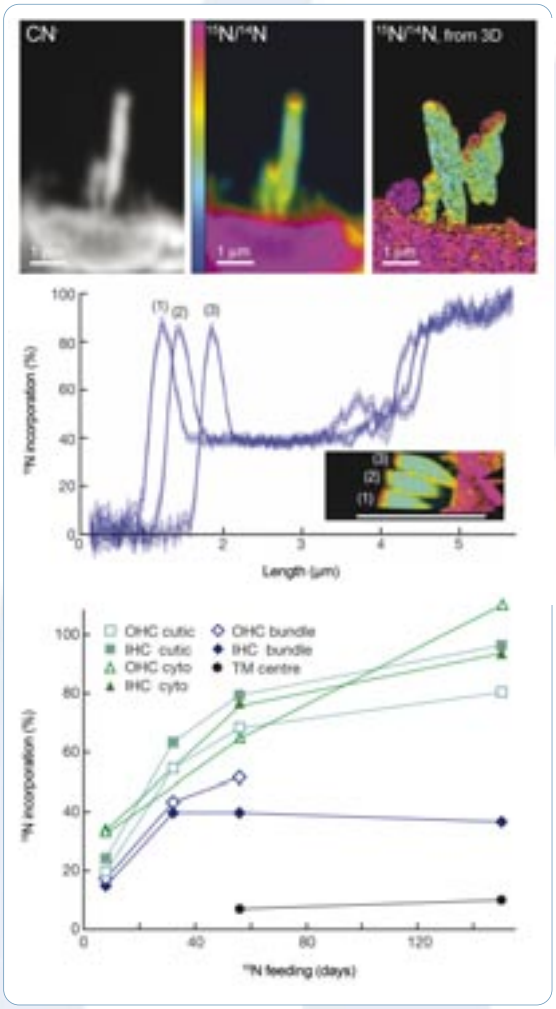
Measuring molecular fluxes with intra-cellular resolution

The NanoSIMS offers a novel, powerful and quantitative means for studying intra- and trans-cellular metabolic pathways, signal transduction, cytoplasmic and nucleo-cytoplasmic translocations, RNA and DNA expression and distribution, vesicular and non-vesicular trafficking, virus penetration, and localization of drugs.

The NanoSIMS can be seen as a sort of "super autoradiography" with unique performance:

- Lateral resolution down to 50nm and depth resolution down to 10-20nm for high resolution 2D and 3D imaging from whole cells or thin microtome sections.
- Possibility to use several tags in parallel or quasi-parallel (e.g. D/H, $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{18}O/^{16}O$). One molecule can be labelled at different positions and/or several molecules can have different labels.
- Very high sensitivity (~X 1000 more than autoradiography)*.

Kinetics of incorporation or turnover of molecules in tissues or cells are accessible through the control of labeling and chase durations. The localized information is derived from pixel signal in small regions of interest inside images.



Localized protein turnover

High resolution 2D & 3D measurements of protein turnover in hair-cell sterocillia from inner ear. Adult mice were fed ^{15}N-leucine. The stereocilia were measured as remarkably stable, incorporating newly synthesized protein at <10% per day. Only stereocilia tips had rapid turnover and no treadmilling process was observed. Large variations of ^{15}N incorporation within the cochlea are observed (ex: tectorial membrane).

From: Multi-isotope imaging mass spectrometry reveals slow protein turnover in hair-cell stereocilia. Duan-Sun Zhang et al. NATURE, vol 481, 26 January 2012.

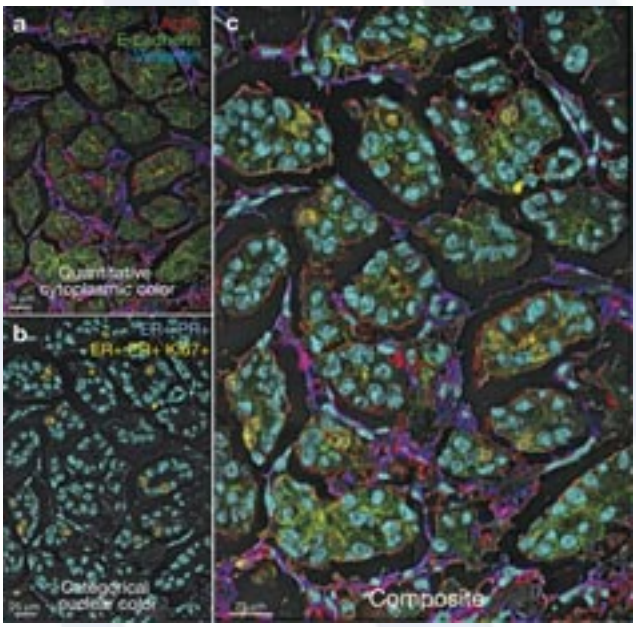
**High-resolution quantitative imaging of mammalian and bacterial cells using stable isotope mass spectrometry. C. Lechene et al. Journal of Biology 2006, 5:20.*

Multiplexed antibody imaging

Immunohistochemistry (IHC) is a tool for visualizing protein expression that is employed as part of the diagnostic workup for the majority of solid tissue malignancies. Existing IHC methods use antibodies tagged with fluorophores or enzyme reporters that generate colored pigments. Because these reporters exhibit spectral and spatial overlap when used simultaneously, multiplexed IHC is not routinely used in clinical settings.

In the example on the right, NanoSIMS was used to image ten antibodies tagged with isotopically pure elemental metal reporters, without overlap in the mass spectrum.

Formalin-fixed, paraffin-embedded human breast tumor tissue sections were stained with ten labels simultaneously. The resulting data validate the principle of multiplexed antibody imaging with the NanoSIMS which provides simultaneously excellent sensitivity, subcellular spatial resolution and high mass resolution. Furthermore, this method can be extended to a few tens of tags in parallel, multiplying the power of antibody intercorrelation. This suggests that this methodology can provide new insights into disease pathogenesis that will be valuable for basic research, drug discovery and clinical diagnostics.



Immunohistochemistry

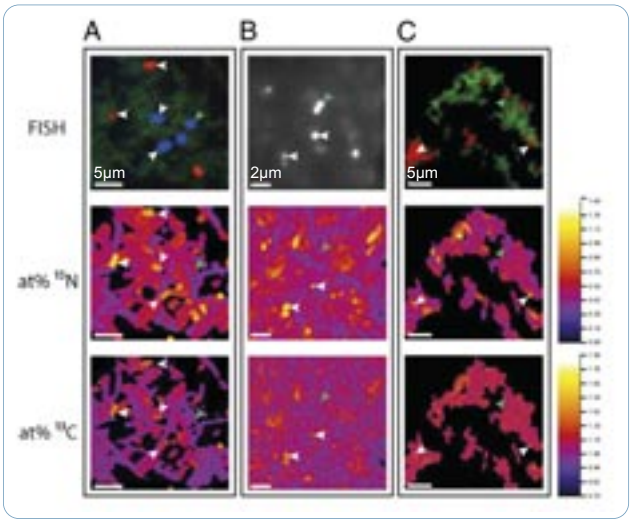
False color rendering of multiplexed antibody imaging. Ten different rare earth elements (REEs) without spectral mass interference between them were conjugated with ten different antibodies.

From: Multiplexed ion beam imaging of human breast tumor. M. Angelo et al. NATURE MEDECINE, 2014 Apr, 20 (4).

Correlative microscopies: NanoSIMS / EM / FISH

In addition to the imaging of seven masses in parallel, the NanoSIMS incorporates in situ optical microscope and SEM capabilities. NanoSIMS data are often correlated with other complementary microscopies, including:

- SEM and TEM, used to document and image the samples with a nm-scale spatial resolution.
- FISH (fluorescent in-situ hybridization), used in microbiology in order to *identify the species* present in the field of view. NanoSIMS measures the *cell metabolism activity* of these identified single cells. Based on stable isotope labelling strategies. Alternatively, FISH fluorophores can be replaced by elemental reporters for one single NanoSIMS measurement of both pieces of information.



From: Host-compound foraging by intestinal microbiota revealed by single-cell stable isotope probing. D. Berry et al. PNAS vol. 110, n°12, March 19, 2013.

Microbiota study

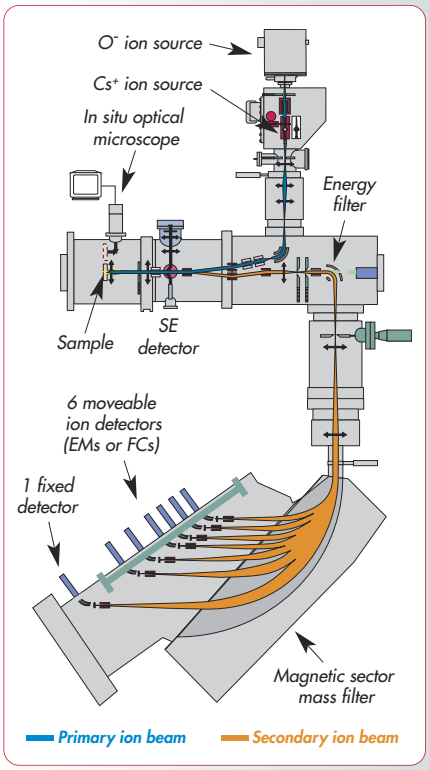
Metabolic host-microbiota interactions in the mammalian gut can be elucidated by combining FISH and NanoSIMS microscopies.

The metabolic activity of bacteria living inside mice intestine, individualy identified with FISH, is measured by supplying mice with 13C- and 15N-threonine amino acid and measuring its incorporation in bacteria through the consumption of the intestine mucus layer. The magnitude and pattern of foraging is shown to depend on the microbiota composition demonstrating the importance of in-vivo studies in complex environments where response can be very different from simplified models.

NanoSIMS (at% ^{13}C & ^{15}N) and FISH images 8 h after i.v. injection of $^{13}C,^{15}N$ threonine.
(A) B. acidifaciens (blue) and Ruminococcaceae OTU_5807 (red) cells
(bar: 5 µm; prepared without embedding; other Bacteria shown in green),
(B) Akkermansia spp. (white) cells (bar: 2 µm; prepared without embedding),
(C) Lactobacillaceae/ Enterococcaceae spp. (red) cells
(bar: 5 µm; prepared without embedding; other Bacteria shown in green).

NanoSIMS: a unique design

The NanoSIMS is based on an original design by Pr. Slodzian, University of Paris Sud, France. Cs^+ or O^- primary ions are focused and rastered on the surface of the sample, at normal incidence. The ions sputtered by this primary beam are collected, and the secondary ion beam is shaped in order to be mass analyzed. Up to seven detectors are available at the exit of the mass analyzer, six of them being moveable. Up to seven ionic species can be simultaneously recorded, originating from the exact same sputtered volume, ensuring reliable isotopic or elemental ratio and excellent distribution comparison.



Synopsis of a CAMECA NanoSIMS 50L

CAMECA is the world premier provider of microanalytical instrumentation. We deliver cutting-edge science and metrology solutions, and offer our customers unparalleled support and maintenance service through the comprehensive AMECARE program.

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