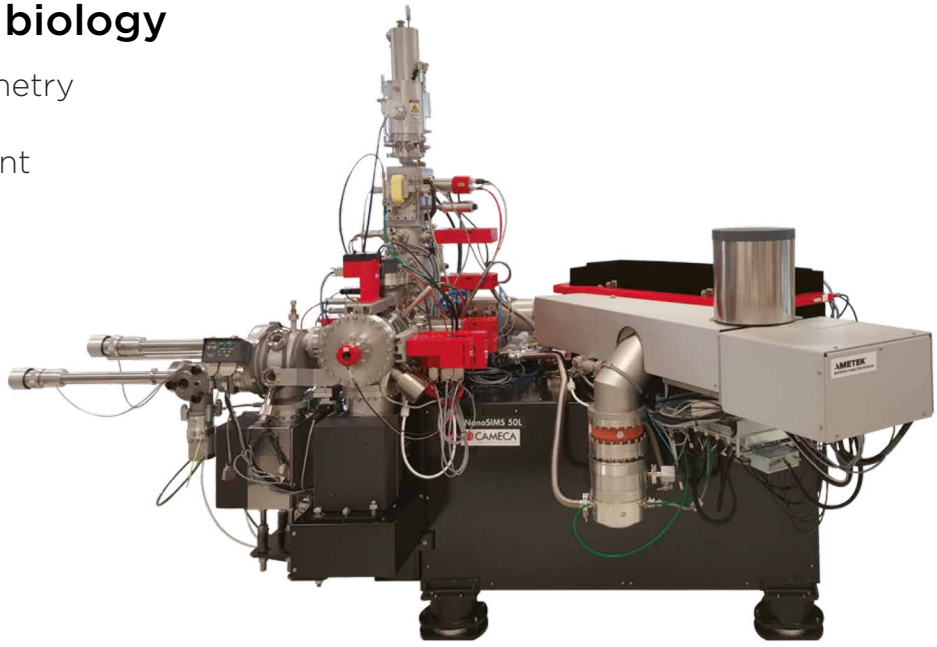
CAMECA
SCIENCE & METROLOGY SOLUTIONS
/ AMETEK

NanoSIMS 50L

A revolution in cellular biology

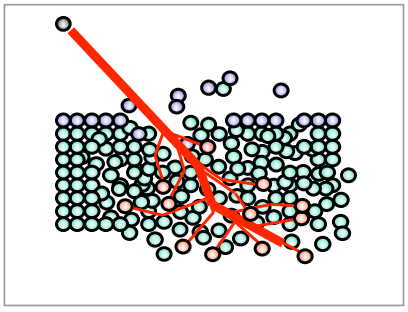
Multiple Isotope Mass Spectrometry
for simultaneous stable isotope
tracer imaging and measurement
at subcellular level



The NanoSIMS: a Scanning Ion Microprobe with a Multicollection Mass Spectrometer

The SIMS technique

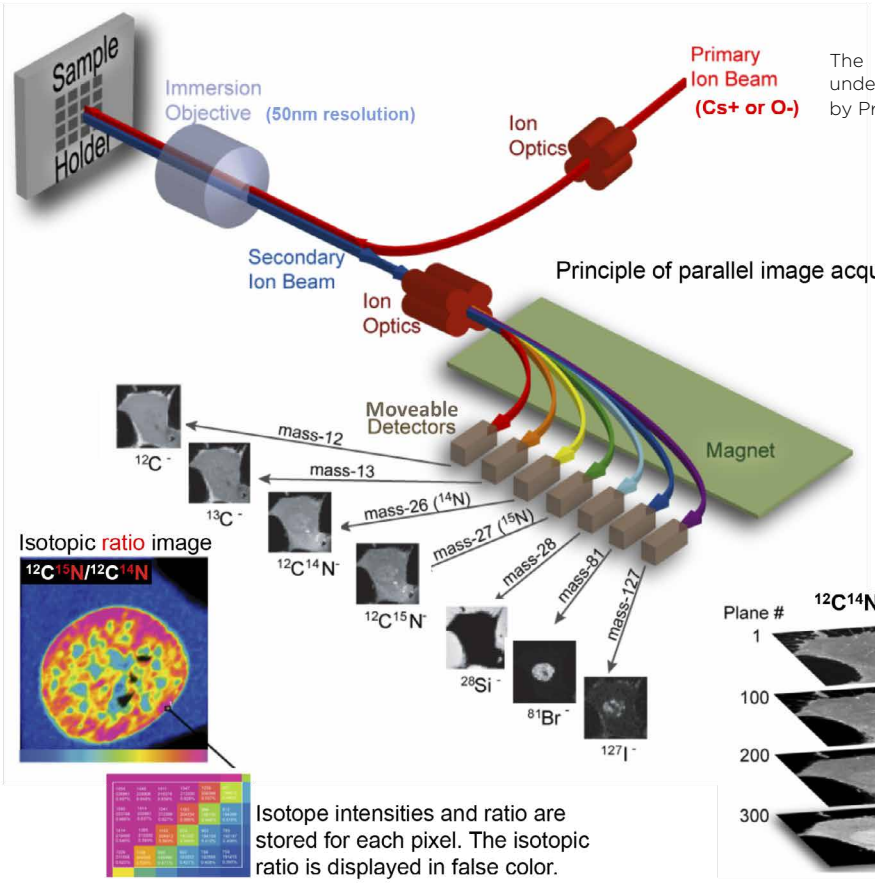
Secondary Ion Mass Spectrometry is based upon the sputtering of a few atomic layers from the surface of a sample induced by a primary ion bombardment. A primary ion impact triggers a cascade of atomic collisions. Atoms and atomic clusters are ejected.
During the ejection process, some atoms and clusters are spontaneously ionized. These secondary ions are a characteristic of the composition of the analyzed area. They are separated according to their mass, and an image containing quantitative information is formed for a selected mass.



SIMS reveals elemental and isotopic surface composition. Characteristics: extreme sensitivity

Description of the NanoSIMS instrument

A DC primary beam of Cs^+ or O^- primary ions is focused and rastered on the surface of the sample, down to 50nm beam size (= lateral resolution). The collected secondary ion beam is shaped in order to be mass filtered by a magnetic sector mass analyzer. Up to seven mass selected images of different elements or isotopes can be simultaneously recorded, ensuring reliable isotopic ratio and perfect image registration.
Each plane image is obtained through the consumption of typically a few nanometers thickness of the sample.The scanning is repeated and a 3D image can be reconstructed from the stack of images.

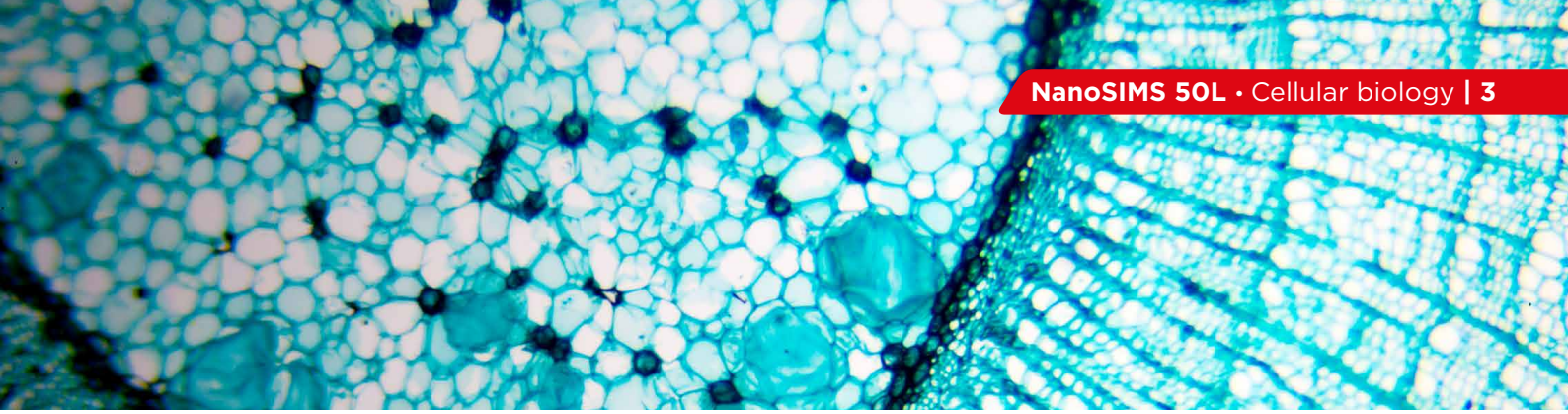


Isotope intensities and ratio are stored for each pixel. The isotopic ratio is displayed in false color.

3D stack of images

The NanoSIMS is manufactured by CAMECA under license from ONERA-UPS. Original design by Pr G. Slodzian, University of Paris Sud

Schematics from: M. Steinhauser et al., NATURE, Vol 481, 26 January 2012



Applications of the NanoSIMS in cellular biology

The main NanoSIMS capabilities

The NanoSIMS permits trace element mapping at intracellular level (50nm spatial resolution). It is an excellent complement to analytical analysis by TEM-EELS/EDS that can provide better lateral resolution (a few nm) on thin sections but often lack elemental sensitivity specially on light elements.

But its main and unique feature is the precise isotopic ratio mapping with 50nm resolution. This can be fruitfully used by coupling it with an experimental strategy of isotope labeling: feeding cells, plants, animals with molecules labeled with one or several stable isotopes (e.g. ^{2}D, ^{15}N, ^{13}C, ^{18}O, ^{33}S, ^{74}Se,...) or radio isotopes (e.g ^{14}C,...). Multiple labeling can be used on the same or different molecules.

What can the NanoSIMS bring to biologist?

The NanoSIMS allows comparison in exactly the same compartments, of images of the distribution and quantitative measurement of the accumulation and fluxes of several labeled molecules. It can be seen as a sort of super autoradiography technique with a much higher spatial resolution (50nm), a much higher sensitivity (around X1000 times more sensitive), a high depth resolution (10-15nm) permitting 3D reconstruction, allowing multiple labeling in parallel, and without the constraints of using radioactive products.

The technique also called Multiple Isotope Imaging Mass Spectrometry (MIMS) offers a novel, powerful and quantitative technique 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.

Why detecting elements and isotopes and not intact molecules?

Identification of molecules can be performed with fluorescence microscopy, with the limitation of resolution and sensitivity brought by optical microscopy and the possible artifacts brought by the use of fluorophore molecules.

The chemical analysis/mapping of large intact macromolecules from the surface of a sample is limited in mass spectrometry by the number of molecules available from the sampling volume and the ionization yield of such molecules.

MALDI permits molecular imaging (ex: allowing the identification and localization of a drug and its metabolites separately) but its lateral resolution is limited to 50-100µm by the ionization yield (although on some set-ups and applications MALDI resolution can be pushed down to a few µm).

TOF-SIMS technique, using a focused primary ion beam instead of a laser as in MALDI, permits to image molecular fragments down to micrometer scale. But subcellular resolution is again prohibited by the too low number of molecules under the spot size and the too low ionization yield of molecules or even fragments.
Also, High Mass Resolution (ex: resolving $^{12}C^{15}N^{-}$ from $^{13}C^{14}N^{-}$) is not possible on TOF-SIMS simultaneously with sub-µm spatial resolution and good enough sensitivity.

The NanoSIMS approach is then to accept to brake the molecules of the sample surface by bombardment with energetic reactive ions (Cs^{+} and O^{-}), detect the more numerous resulting atomic ions and make use of isotope labeling experiments by measuring isotopic ratios with high mass resolution and 50nm resolution.

Recommended detailed introduction to the method: A High-resolution quantitative imaging of mammalian and bacterial cells using stable isotope mass spectrometry. Claude Lechene et al. Journal of Biology 2006, Vol. 5, article 20.

Sample preparation and mounting

The sample is analyzed at room temperature under Ultra High Vacuum (range low E-9 to high E-11 mbar). Similar to TEM analysis, it must be dehydrated and generally fixated.

Flat samples are required. Thin sections (0.3-0.5 µm) are preferable to avoid sample charging under ion bombardment. Sample preparation will then depend on application:

- Most commonly chemical fixation, resin embedding, thin section (0.3-1µm) deposited on metal or silicon substrate.
- Fast freezing, cryo-substitution with resin embedding and thin section.
- Fast freezing, sandwich-fracture of cultured cells, then freeze-drying.
- For bone sections, resin embedding followed by mechanical polishing can be used.



Example of 2-inch diameter sample holder: here the thin sections are deposited on 5x5mm silicon pieces.



The sample holder is introduced in the load-lock. The door is closed and the load-lock pumped. The sample holder is transferred to the intermediate storage chamber (up to 7 sample holders). The sample holder can then be transferred in the analysis chamber. Each chamber is individually pumped and isolated with valves.



Thin tissue sections mounted on 5x 5mm silicon piece.

Photos of the NanoSIMS sample introduction system by courtesy of C. Lechene, Harvard Medical School, Brigham & Women's Hospital (NRIMS, NIH), Boston, MA., USA.

Melanoma in mouse lung imaged through the Iodine of BZA

The 50nm spatial resolution provides unambiguous localization. Multiple labelings on the same molecule allows NanoSIMS to better differentiate an original molecule from its metabolites.

BZA localized through I^- is well targeting the Melanoma localized through CN^-.

The N-(2-diethylaminoethyl)-4-iodobenzamide or BZA, developed in INSERM Unit 484, Clermont-Ferrand, France, is a melanoma imaging agent for the diagnosis of malignant melanoma and metastases.

A phase II scintigraphic clinical trail of iodine-123 BZA on a total of 110 patients proved the usefulness of this agent for the detection and follow-up of patients with melanoma, with a diagnostic sensitivity of 81%, an accuracy of 87% and a specificity of 100%. The melanin affinity of this compound was assessed with melanoma cells or synthetic melanin models: the results showed that BZA-melanin binding is a complex process in which various bindings may participate, such as ionic and hydrophobic interactions.

The study of the localization of BZA in melanotic cells by SIMS was essential as a new step to a better understanding of the binding mechanism of BZA to melanin.

For this present work, the model used was B16 murine melanoma. The cell line was maintained as monolayer in tissue culture minimum essential medium supplemented with 10% fetal calf serum and antibiotics, and passaged by trypsinisation.

Resuspended in phosphate buffer saline, cells (0.2x106 /200µl) were injected intravenously to male mice.

Twelve days later, pulmonary colonies of B16 were visible. At this step, BZA (1mg/200µl) was administered intravenously to mice bearing pulmonary colonies. After 6 hours, animals were sacrificed and lungs were taken.

After dissection and cryo-preparation, images were recorded in parallel with the NanoSIMS 50:

- ^{26}CN (black and white) for histological overview, ^{31}P (in yellow), ^{127}I (in red) from BZA
- Fourth image is an overlay of ^{127}I image in red and ^{31}P image in yellow.

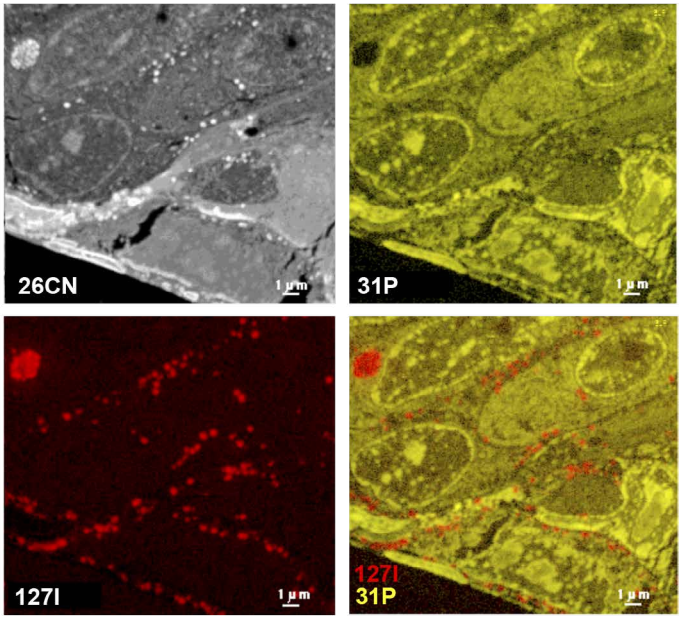


Image #1
Field: 20 x 20 microns. Acq. time: 20min.

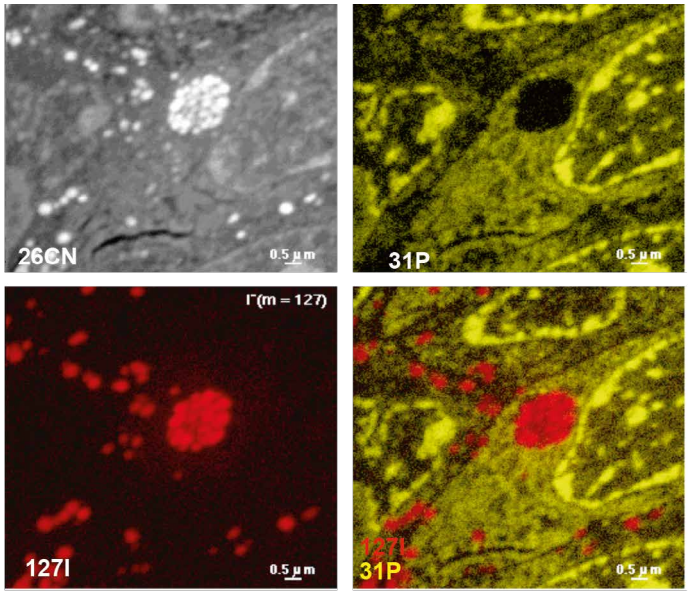


Image #2
Recorded on upper left part of image #1.
Field: 10 x 10 microns. Acq. time: 20min.

See: Ultra-structural cell distribution of the melanoma marker iodobenzamide: improved potentiality of SIMS imaging in life sciences. Jean-Luc Guerquin-Kern, F. Hillion, J-C Madelmont, P. Labarre, J. Papon, A. Croisy. BioMedical Engineering OnLine 2004, 3:10

Mapping BrdU DNA replication marker to follow kidney tissue repair after renal ischemia

NanoSIMS is used to help identify the cell type(s) at the origin of regeneration after renal ischemia, their location in the kidney, their abundance and their migratory capability.

After renal ischemic injury, there is death of renal tubular cells. Regeneration may occur. Which cell type will divide and differentiate into the specialized renal epithelium is unknown. This knowledge is important both for basic cellular biology and for therapeutic design after renal ischemia.

The study performed here is designed to identify the cell type(s) at the origin of regeneration after renal ischemia, their location in the kidney, their abundance and their migratory capability.

The images are from one of the experimental protocols used:

- at day 0, first surgery of 10 weeks mice: they are sham operated, let recover from surgery and their renal function is followed for 7 days.
- at day 8, second surgery to create a 30 minutes renal ischemia. BrdU (bromo-deoxyuridine, a DNA replication marker which will incorporate into the DNA of cell undergoing division-regeneration) is injected intra-peritoneally 40 and 46 hours after the renal ischemia.
- at 48-50 hours after 2d surgery, the kidneys are chemically fixed by direct intra arterial perfusion and then prepared for MIMS analysis (dehydratation, Epon embeding, thin sectioning).

The NanoSIMS allows to record three images in parallel:

- ^{26}CN for histological overview
- ^{31}P for identification of cellular nuclei
- ^{81}Br (from BrdU) for identification of the cells which had nuclei undergoing DNA replication i.e. potentially dividing cells.

Colored figure is an overlay of images: ^{26}CN in Red, ^{81}Br in Green and ^{31}P in Blue.

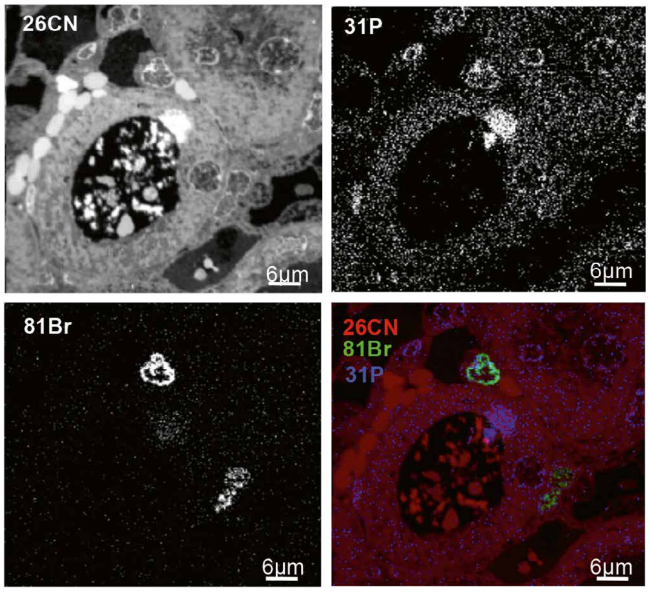


Image #1
Field: 60 x 60 microns, 20 min acq. time, 16keV Cs+, negative secondary ions

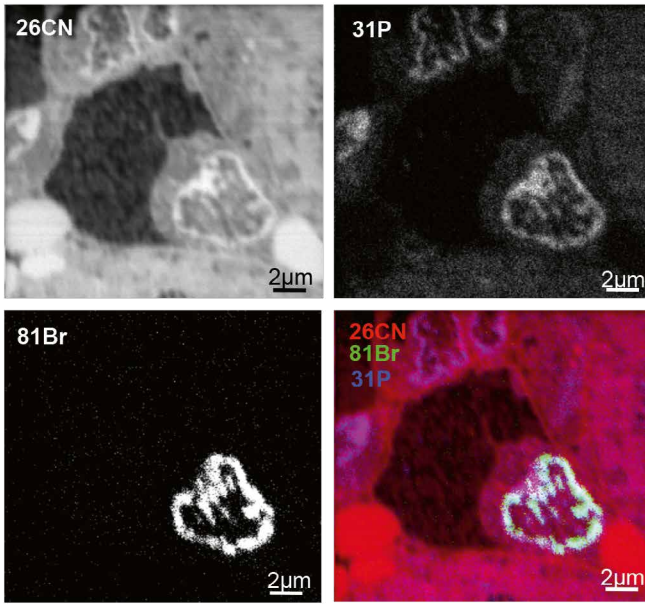


Image #2
Field: 20 x 20 microns, 10 min acq. time, 16keV Cs+, negative secondary ions

Courtesy: C. Lechene, Harvard Medical School, Brigham and Women's Hospital, Boston, MA. USA., JV. Bonventre, Massachusset General Hospital

Cosmetics: incorporation of a molecule in hair grown in-vitro

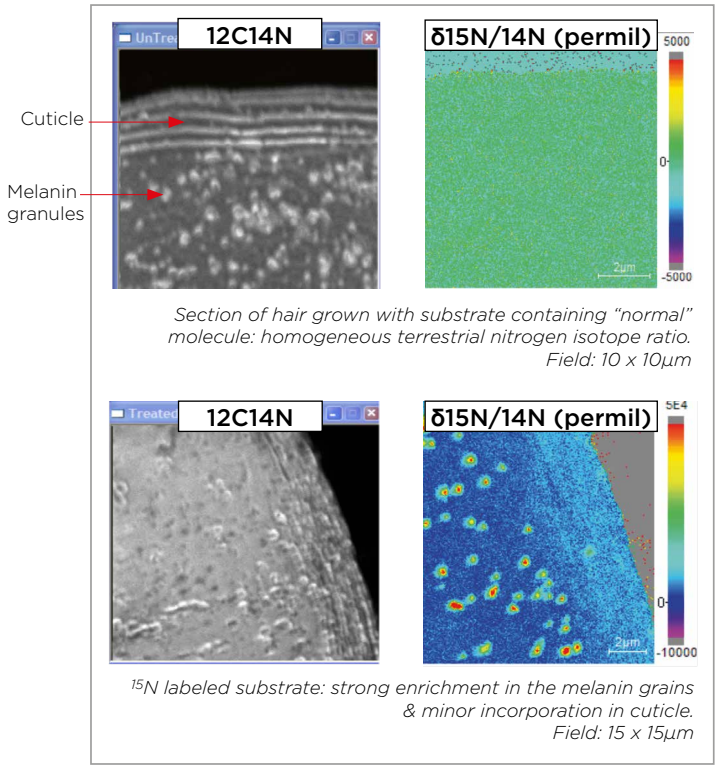
The NanoSIMS is used in cosmetics R&Ds (hair treatments) and dermatology (skin, creams) in order to measure molecule incorporation in tissue though the isotope ratio imaging of sections.

For hair, sectioning in the longitudinal axis (not shown here) permits to follow the incorporation of a molecule from the living bulb to the growing part of the hair.

Nitrogen labeling

The molecule incorporation is measured by the isotope ratio deviation from the terrestrial standard value expressed in permil. Note the huge values of enrichment illustrating the sensitivity of the method.

Nitrogen signal is measured in biological tissues through CN^- ion instead of N^+ due to the much higher ionization yield of the former.



Section of hair grown with substrate containing "normal" molecule: homogeneous terrestrial nitrogen isotope ratio. Field: 10 x 10µm

^{15}N labeled substrate: strong enrichment in the melanin grains & minor incorporation in cuticle. Field: 15 x 15µm

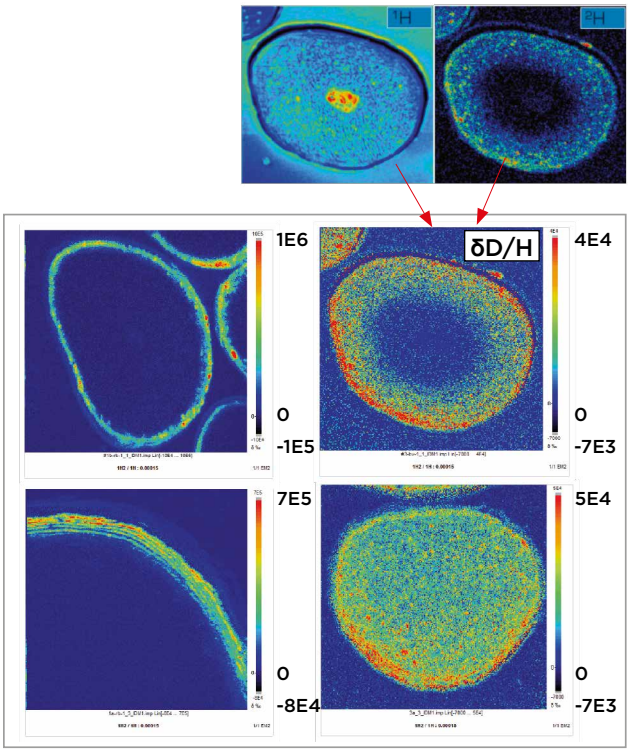
Deuterium labeling

Different examples of cross sections of hair grown in medium enriched with deuterium-labeled chemical compound.

Biological samples or even microtomed sections are not always perfectly flat.

The NanoSIMS data are quite insensitive to this factor through:

- the use of a **normal incidence** primary ion bombardment and a **co-axial** secondary ion collection ensuring **minimal shadowing**,
- the isotope ratio measurement: based on the **normalization** of two images, it ensures minimal topographic influence.



Samples cryo-processed according to P. Hallégot, J-N. Audinot, H.-N. Migeon, Appl. Surf. Sci. 252 (2006) 6706-6708.

Melanoma cell: simultaneous imaging of ^{14}C and ^{15}N isotopic markers

The parallel detection of the NanoSIMS allows the simultaneous intra-cellular isotopic ratio imaging and measurement.

Multiple simultaneous labeling (radioactive and/or stable isotopes) is possible allowing linking with autoradiography protocols used at larger scale.

Tissue sections (0.5 micron thick) of a culture of melanoma cells incubated 24 hours with two labeled molecules ^{14}C-Uridine (1 mM) and ^{15}N-Arginine (1mM), were analyzed.

The preparation was fixed with glutaraldehyde and embedded in epoxy resin.

$^{12}C^-$, $^{14}C^-$, $^{12}C^{14}N^-$ and $^{12}C^{15}N^-$ ion image were simultaneously collected with a mass resolving power of 5000.

The different cell constituents are seen on the $^{12}C^{14}N^-$ image (lower-left).

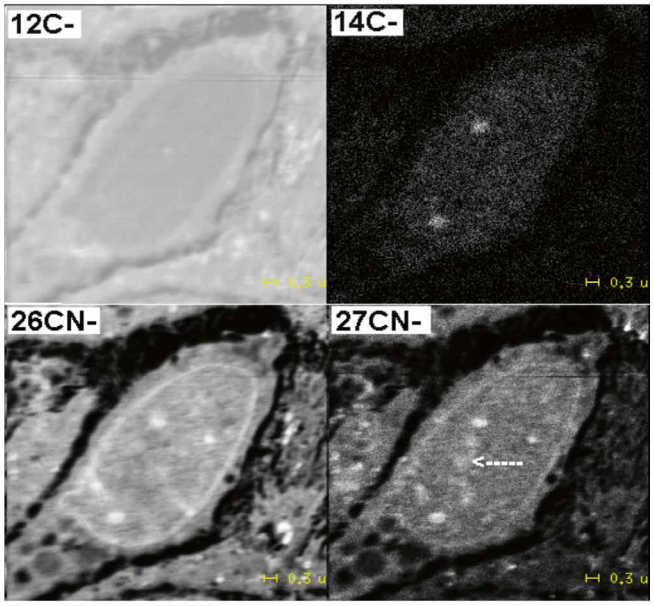
The nuclear membrane is clearly visible. The $^{14}C^-$ image (upper-right) shows the distribution of ^{14}C-Uridine which is present in the cytoplasm and in the nucleus, with a higher concentration in the nucleoli (two bright dots) as expected.

Distribution of ^{15}N (both natural and linked with Arginine) is shown on the $^{12}C^{15}N^-$ image (lower-right).

Differences between ^{14}N image and ^{15}N image would correspond to incorporated ^{15}N-Arginine: higher incorporation seems to occur in some nuclear structures (white arrows) which could correspond to newly-formed nuclear proteins (Histones).

At the opposite the nuclear membrane contrast reveals a lower rate of incorporation of ^{15}N-Arginine.

Simultaneous acquisitions allow to estimate (i) the concentration of ^{14}C-U by calculating the isotopic ratio $^{14}C/^{12}C$, (ii) the renewal rate of ^{15}N-A by calculating the increase of the isotopic ratio $^{15}N/^{14}N$ above the natural occurrence (0.367%).



Field: 10µm x 10µm
Cs 16 keV, 2pA
Spot size: 0.1 µm
Mass Resolution = 5000
($^{12}C^{15}N^-$ $^{13}C^{14}N$ interference resolved)
Acq. time: 10 min.
Linear grey scale
Parallel imaging

Note the quality and lateral resolution of the mass filtered images (ex: membrane).

Courtesy of Elif Hindié, Laboratoire de Biophysique, Faculté de Médecine, Université Paris-XII, Créteil France.

Intracellular transport of STxB (B sub-unit of Shiga toxin)

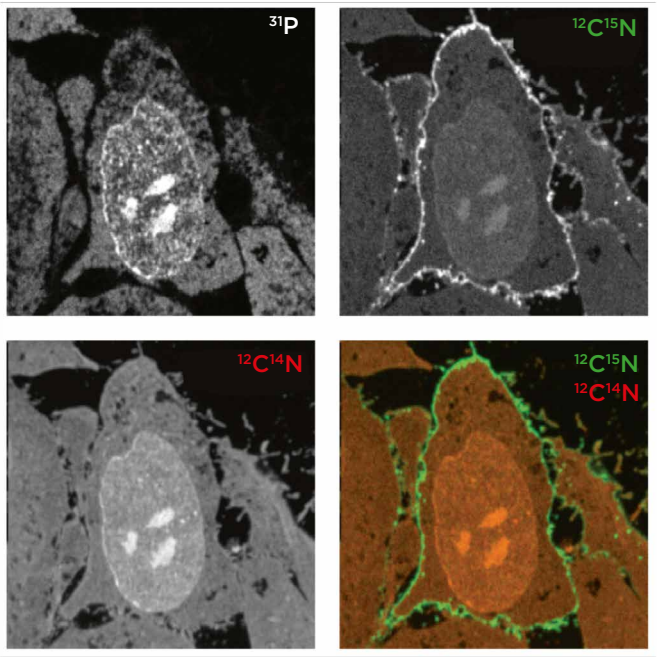
The NanoSIMS permits sub-cellular detection of isotopically labeled, exogenous molecules and imaging of their transport pathways at high mass and spatial resolution. The use of stable isotopes as markers can replace the large and chemically complex tags used for fluorescence microscopy and thus avoids alterating the molecule's chemical and physical properties.

Exogenous peptide vector with potential use in medical therapy HeLa human cancer cells (with membrane receptor).

Labeling:
The ^{15}N-labeled ShigatoxinB subunits were eluted by glycine buffer (pH2.2) and estimated to be 99% pure by Tris–Tricine gel electrophoresis.

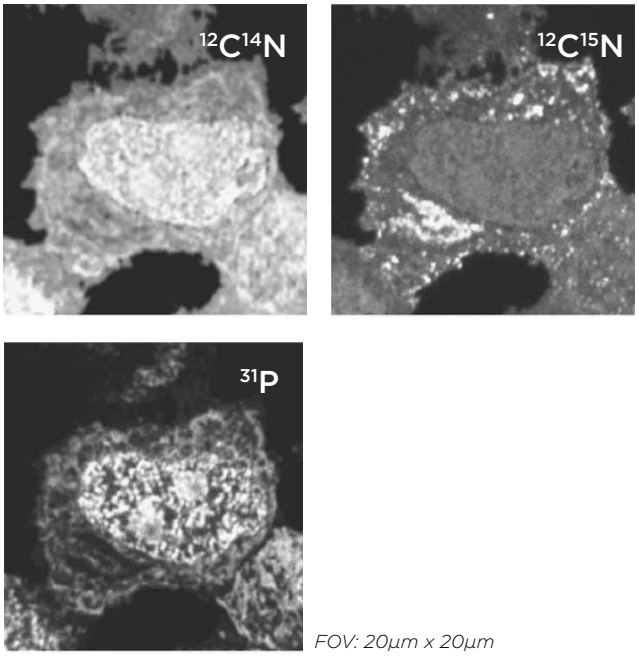
Preparation:
Chemical fixation with glutaraldehyde and osmium tetroxide (OsO4). Embedding in Spurr's resin (low nitrogen level). 0.1µm ultramicrotomy.

MEMBRANE BINDING (right):
30min at 0°C

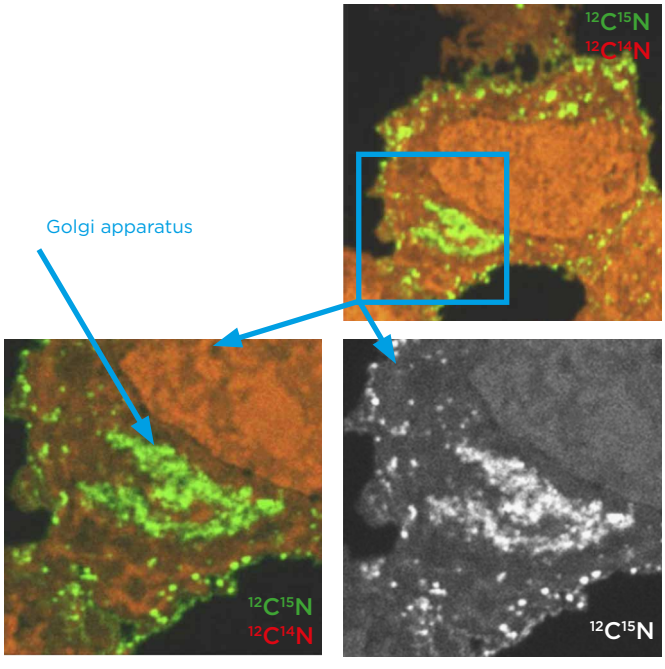


FOV: 25µm x 25µm

INTERNALISATION (below):
after incubation at 37°C for 40min



FOV: 20µm x 20µm



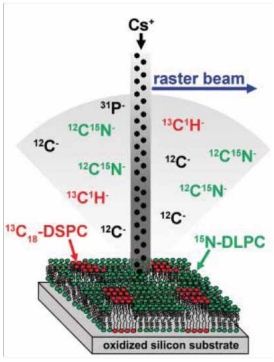
FOV: 10µm x 10µm

Sub-cellular localisation of a 15N-labelled peptide vector using NanoSIMS imaging. Winfried Röhmer, Ting-Di Wu, Patricia Duchambon, Mohamed Amessou, Daniele Carrez, Ludger Johannes, Jean-Luc Guerquin-Kern, Applied Surface Science 252 (2006) 6925-6930

Phase separation of lipid membranes

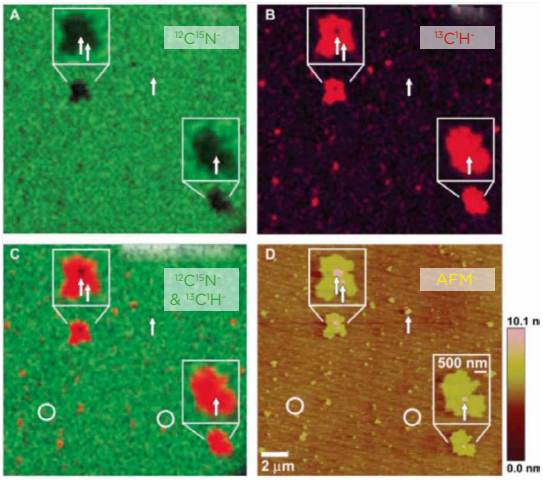
Lipid composition within small regions (~100nm) of the bilayer membrane were quantified using a stable isotope labeling strategy. Correlation with AFM (or other high resolution microscopy techniques) can bring complementary informations.

Lateral variations in membrane composition are postulated to play a central role in many cellular events, but it has been difficult to probe membrane composition and organization on length scales of tens to hundreds of nanometers. We present a high-resolution imaging secondary ion mass spectrometry technique to reveal the lipid distribution within a phase-separated membrane with a lateral resolution of ~100 nanometers. Quantitative information about the chemical composition within small lipid domains was obtained with the use of isotopic labels to identify each molecular species. Composition variations were detected within some domains.



Schematic showing NanoSIMS analysis of phase-separated lipid bilayer (not to scale):

At room temperature, gel and fluid phases, mostly composed of ^{13}C18-DSPC (red) and ^{15}N-DLPC (green), respectively, are present in the bilayer. The gel phase is ~2 nm higher than the neighboring fluid phase and can be imaged by AFM (compare with Fig. 2D). The sample is freeze-dried to preserve the lateral organization within the bilayer and analyzed with the NanoSIMS. The secondary ions that are characteristic of $^{13}C_{18}$-DSPC and ^{15}N-DLPC ($^{13}C^1H^-$ and $^{12}C^{15}N^-$, respectively) are used to identify each component in the NanoSIMS image.

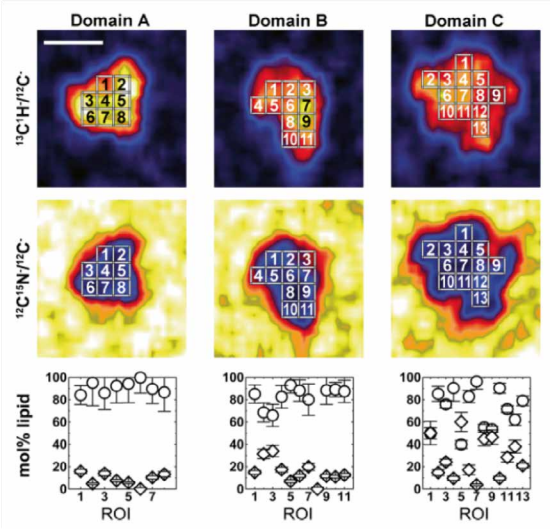


Phase-separated supported lipid bilayer that was freeze-dried and imaged by NanoSIMS and AFM.

(A to C) NanoSIMS images of the normalized $^{12}C^{15}N^-$ signal that localizes ^{15}N-DLPC (A), the $^{13}C^1H^-$ signal that localizes ^{13}C-DSPC (B), and the overlaid $^{12}C^{15}N^-$ and $^{13}C^1H^-$ signals (C). (D) AFM image of the same region before NanoSIMS analysis. The contrast levels within the NanoSIMS images reflect the normalized signal intensity, corresponding to 100 and 0 mol % of the appropriate isotopically labeled lipid, as determined from calibration curves. Arrows indicate objects in the AFM images that are unlabeled debris, not domains. Domains with diameters as small as ~100 nm, as measured by AFM, were visible in the SIMS images (e.g., those highlighted with circles).

Quantitative analysis of gel-phase domains.

Each gel-phase domain was divided into regions of 3 pixels by 3 pixels; specific regions of interest (ROIs) within each domain are shown on the NanoSIMS images. The graphs illustrate the amount of $^{13}C_{18}$-DSPC (o) and ^{15}N-DLPC (◊) detected within each domain for the numbered ROI, where each data point represents a region of 3 pixels by 3 pixels within a domain, and the error bars represent the uncertainty calculated from counting statistics. Statistically significant lateral variations in lipid composition were detected in domain C, where ROIs 5, 8, and 9 are in the vicinity of the fluid-phase subdomain that was detected by AFM (domain C, circled region).



From: Mary L. Kraft, Peter K. Weber, Marjorie L. Longo, Ian D. Hutcheon, Steven G.Boxer, SCIENCE, vol. 313, 29 sept 2006

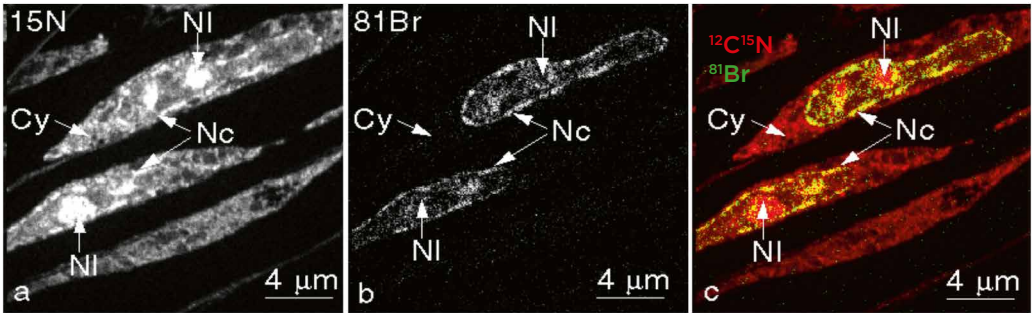
RNA and DNA colocalization by pulsing rat embryo fibroblasts with ^{15}N-uridine and bromo-deoxyuridine

Simultaneous imaging of newly synthesized RNA and DNA localized with ^{15}N and ^{81}Br marker ions. Internal control available from non-labeled isotopes and embedding media.

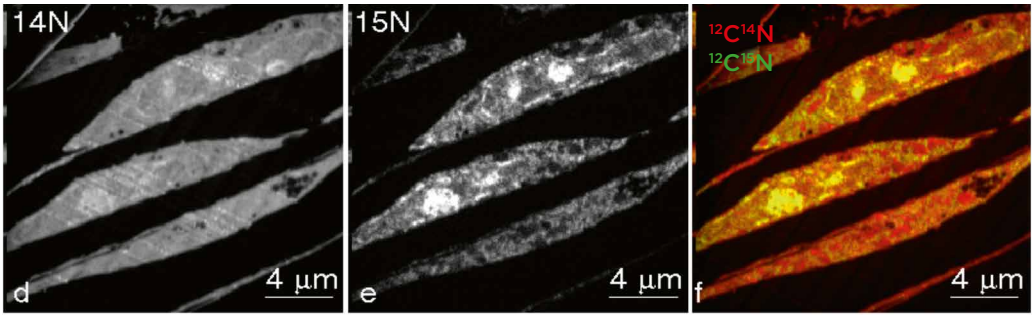
Rat embryo fibroblasts were pulsed with 15N-uridine and BrdU as markers of newly synthesized RNA and DNA, respectively. The NanoSIMS 50L permits to image simultaneously ^{12}C, ^{13}C, ^{14}N, ^{15}N (signing new DNA) and ^{81}Br (signing new RNA).

Sample preparation: fixation (formaldehyde, glutaraldehyde, picric acid), Epon embedding, sectioning at 0.5 µm thickness, mounting on polished stainless steel substrate.

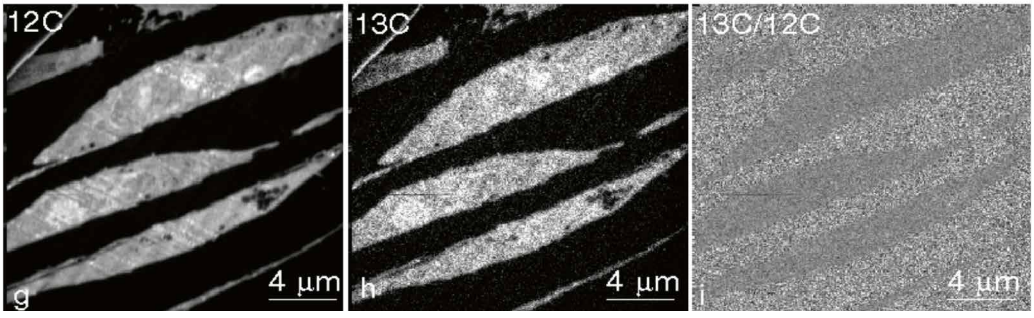
Cy: cytoplasm , Nl: nucleoli , Nc: cell nucleus. FOV: 20µm x 20µm



First row: *colocalization of newly synthesized, BrdU tagged DNA and newly synthesized, ^{15}N-uridine tagged RNA. The overlap between them shows up as yellow*



2nd row: *newly synthesized RNA is localized (through the excess of ^{15}N isotope) in the nucleoli, along the nuclear envelope and in the cytoplasm of the top cell.*



3rd row: *the absence of intracellular contrast in the carbon isotope ratio image validates the highly contrasted ^{15}N/^{14}N data.*

See: A High-resolution quantitative imaging of mammalian and bacterial cells using stable isotope mass spectrometry. Claude Lechene et al. Journal of Biology 2006, Vol. 5, article 20.

Localized protein turnover of cochlea hair cells from adult bullfrogs fed ^{15}N-leucine

Problem of protein turnover in hair cells (a hair cell is forever)

- Hair cells don't regenerate in mammals, and must live for decades in human.
- Hair cell proteins must turn over to replace normal degradation and traumatic damage.
- Hair bundle morphology must be maintained while proteins are replaced.

General experiments

Adult bullfrogs
Fed ^{15}N-Leucine food 1/day
Sacrificed at 1, 2, 4, 8, 16, 22, 32 days
Inner ear dissected out, fixed Plastic embedding followed by ultrathin sections.
Saccular hair cells analyzed by MIMS.

Localized, quantitative isotope ratio measurement extracted from regions of interests (ROIs) :

The two images of $^{12}C^{14}N$ and $^{12}C^{15}N$ are recorded in parallel.
The $^{12}C^{15}N$ image is then divided pixel by pixel by the $^{12}C^{14}N$ image to obtain the ratio image.

The isotope ratio of each pixel is displayed in false color, here from blue = terrestrial $^{15}N/^{14}N$ to pink = enrichment in $^{15}N/^{14}N$, guiding the definition of small regions of interest (ROIs).

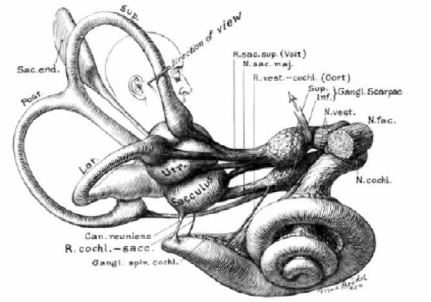
All pixel intensities from each ROI are summed in order to increase the statistics of the isotopic ratios.

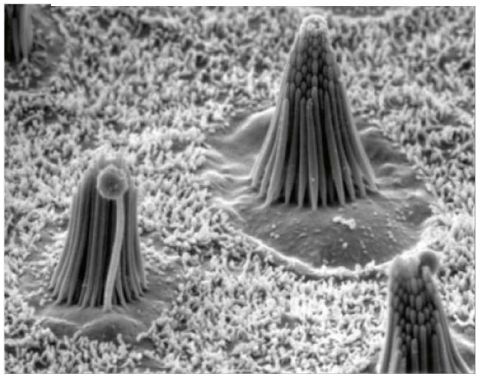
The images are generally acquired in multiple image planes, each plane coming from the sputtering of a few nanometer thickness of the sample.

The plane images are corrected for sample drift correction in case of long acquisition time. The multiple plane images can then be:

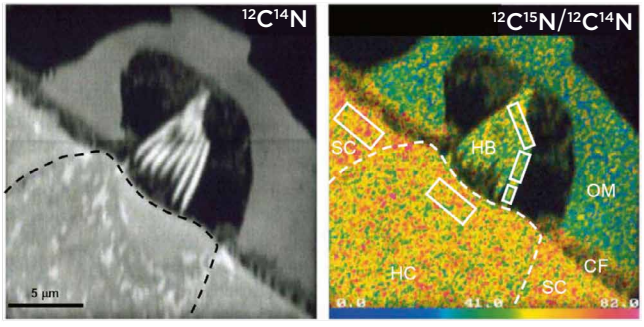
- added without loss of lateral resolution in order to increase the statistics
- or stacked and visualized in 3D for a better understanding of the depth and spatial distribution.

→ Quantitative nitrogen renewal (= protein turnover) with statistical error bars can be quantitatively assigned to very small specific areas.

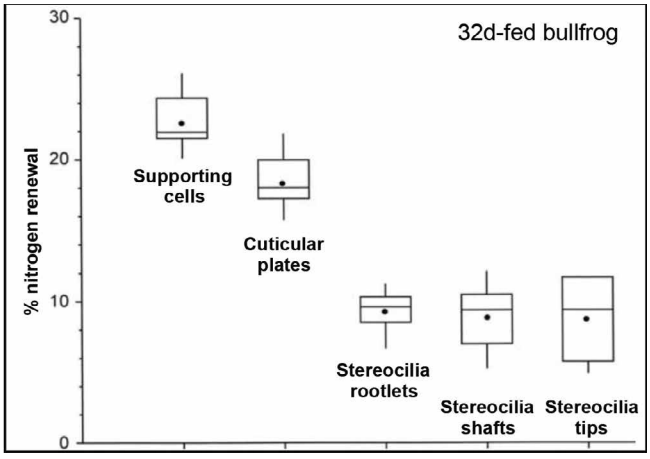




SEM images of frog saccule with stereocilia



32 days 15N-Leucine feeding. HC: hair cell; SC: supporting cell; OM: otolithic membrane; CF: cuticular floor ; HB: hair bundle.

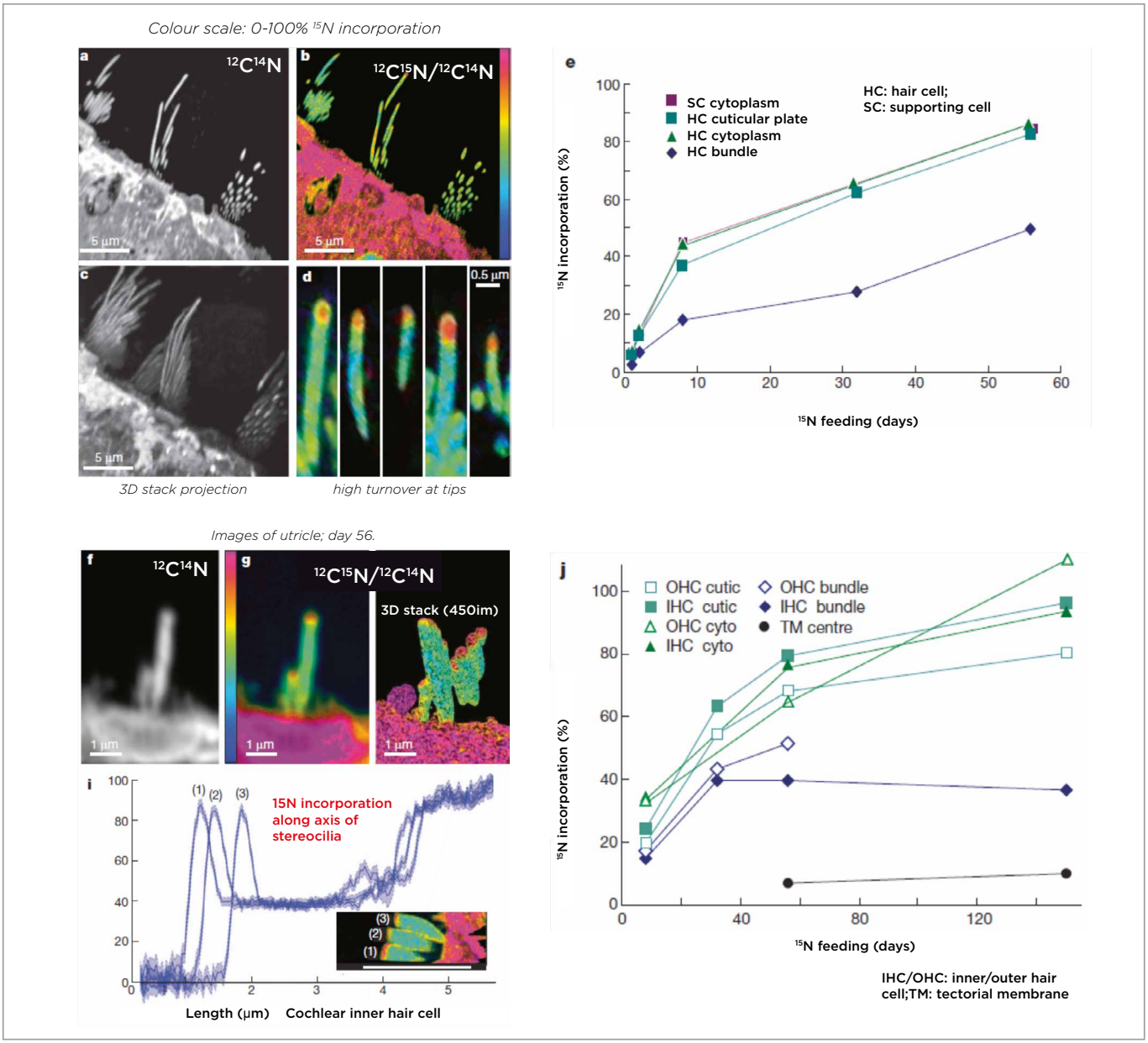


From: of D.P. Corey & C. Lechene, Howard Hughes Medical Institute, Harvard Medical School & Brigham and Women's Hospital, USA.

Slow protein turnover in mouse hair-cell stereocilia: Incorporation of ^{15}N-leucine into adult mouse hair cells

When coupled with a strategy of isotopic labeling the NanoSIMS permits quantitative measurement of molecule turnover with a spatial resolution better than 100nm together with 3D visualization.

The concept of analysis described in the former page was applied extensively to adult frogs and mice and to neonatal mice, in vivo and in vitro. 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.



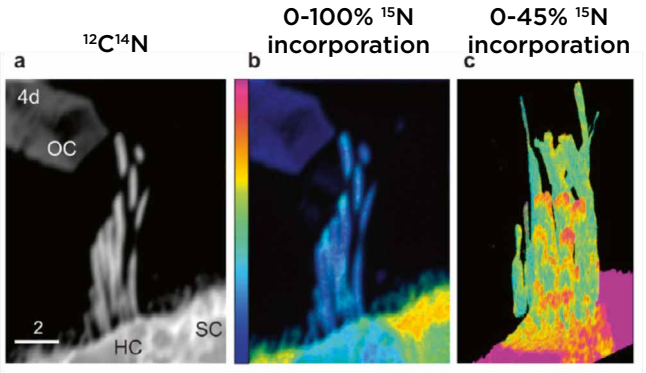
From: Multi-isotope imaging mass spectrometry reveals slow protein turnover in hair-cell stereocilia.
Duan-Sun Zhang et al. NATURE, vol 481 , 26 January 2012. 3D movies are freely available as supplements from Nature website.

Slow protein turnover in hair-cell stereocilia: neo-natal mouse utricle

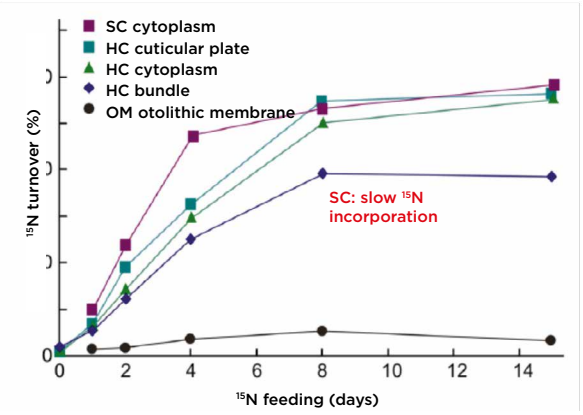
The NanoSIMS permits 50nm lateral resolution and 10-15nm depth resolution in most materials. It is of course possible to combine multiple labeling experiments.

The concept of analysis described in the two former pages was applied to neonatal mice, in vivo, in order to measure protein incorporation in or loss from the stereocilia. The technique of isotope labeling permits an infinite number of experimental possibilities for studying incorporation, loss or trafficking of given molecules or classes of molecules. 3D visualization is possible by stacking individual planes of isotopic ratio images. 3D movies from this article are freely available as supplements from Nature website.

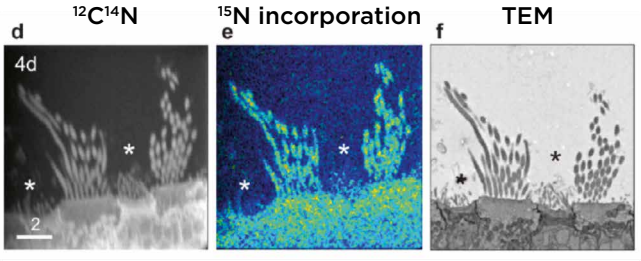
Experiment of ^{15}N incorporation



Pup nursed on ^{15}N milk from birth, after 4 days



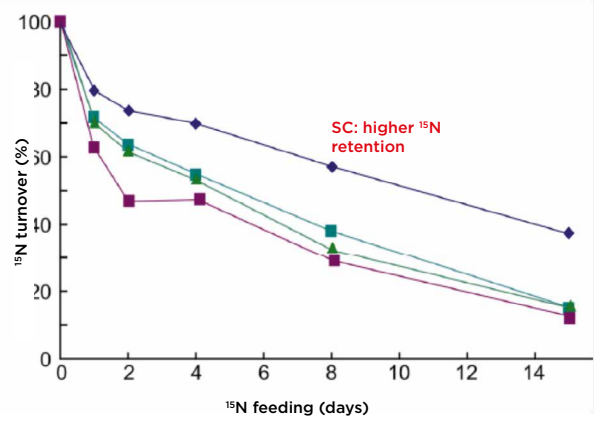
Experiment of ^{15}N loss



Hair cells from a pup born to a ^{15}N mother and nursed on ^{14}N milk from birth, after 4 days.

The left/center and right images were recordred on adjacent microtome sections.

Combining with TEM imaging permits to correlate the protein turnover mapping from NanoSIMS with the structural information given with TEM with a better lateral resolution.



Multi-isotope imaging mass spectrometry reveals slow proteinturnover in hair-cell stereocilia.
Duan-Sun Zhang et al. NATURE, vol 481 , 26 January 2012. 3D movies are freely available as supplements from Nature website.

Measurement of in vivo lipid turnover at high resolution within individual Drosophila lipid droplets

Combining with ^{13}C labeling experiments, precise measurements of in vivo lipid turnover are possible at high resolution within individual Drosophila lipid droplets. The NanoSIMS thus allows the use of very small model systems inaccessible by other techniques.

Drosophila provides a model system in which to study the genetics of lipid metabolism, in vivo. But due to its small size it can be difficult to purify sufficient amounts of any one cell type to do conventional mass spectrometry. The NanoSIMS sensitivity and spatial resolution can solve this problem. Here the NanoSIMS was used to measure and compare the incorporation and turnover of diet-derived 13C-palmitate between cells of the anterior midgut and from fat body adipocytes.

Figure 1: Drosophila anterior midgut enterocytes accumulate more ^{13}C from 24-h exposure to dietary ^{13}C-palmitate than adipocytes (fat body cells (fb). Lipid droplets (arrows) have low nitrogen content and so appear dark in ^{14}N images (top panels). ^{13}C incorporation into lipid droplets shown in $^{13}C/^{12}C$ images (same color scale).

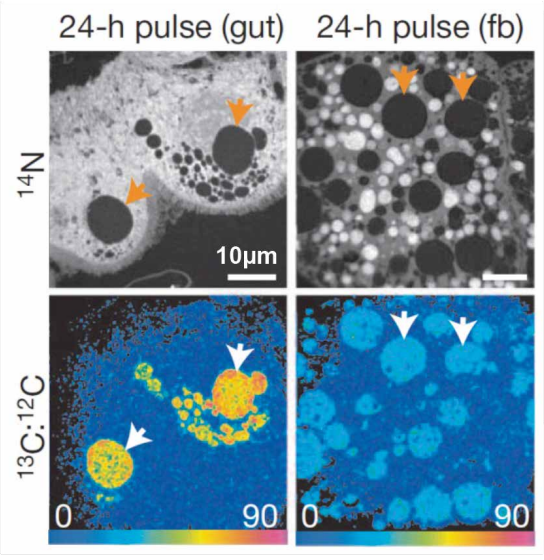


Figure 3: Rapid lipid turnover in Drosophila anterior midgut enterocytes (gut) after larval dietary ^{13}C-palmitate pulse-chase. Strong ^{13}C incorporation at the end of the pulse (bottom left) and rapid elimination during a 4-h chase (bottom right).

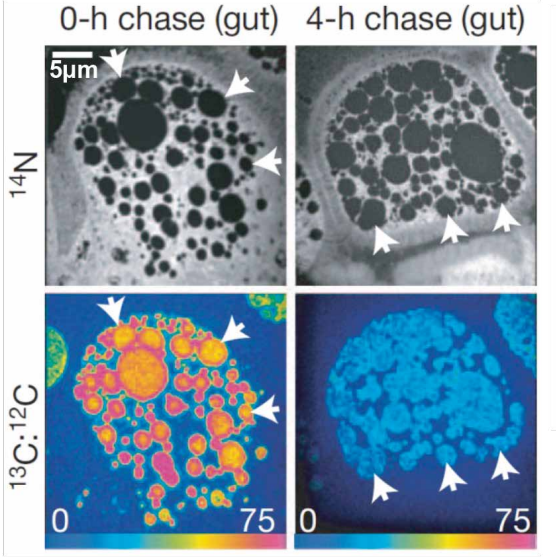


Figure 2:
Time course of ^{13}C accumulation in Drosophila lipid droplets of anterior midgut enterocytes (Gut) and adipocytes (Fat body) over 24 h of larval exposure to dietary ^{13}C-palmitate.

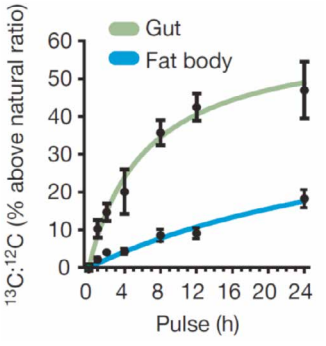
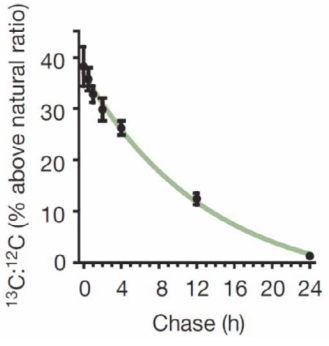


Figure 4:
^{13}C tracer dilution in the lipid droplets of anterior midgut enterocytes during a label-free chase fits an exponential decay curve (R^2 = 0.90, half-life = 9.1 h).



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

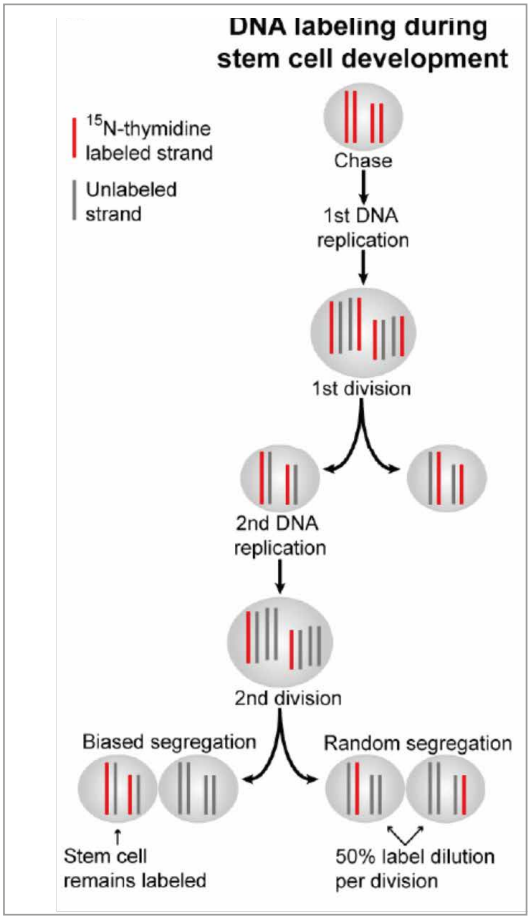
Stem cell division and the immortal strand hypothesis (1/3)

The "immortal strand" hypothesis predicts that during asymmetric stem cell division chromosomes containing older template DNA are segregated to the daughter destined to remain a stem cell, thus insuring lifetime genetic stability. After validation of the method in-vitro and in-vivo, this hypothesis was tested with experiments involving stable isotope labeling and quantitative MIMS analysis with the NanoSIMS.

Experiments of DNA labeling during stem cell development:

Strands that are labeled at the time of stem cell formation can undergo either random or biased segregation during subsequent divisions. Biased DNA strand segregation leads to long-term label retention. Random DNA strand segregation results in the equal distribution of labeled DNA strands to each daughter cell and thus a 50% dilution of label with each division.

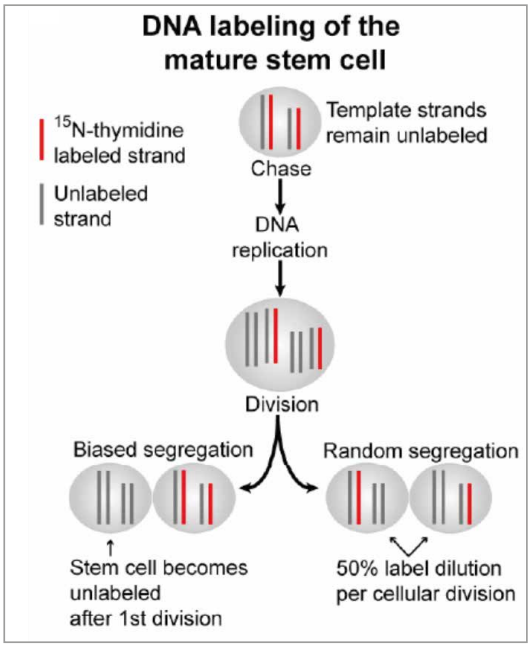
→ "After labeling mice with ^{15}N-thymidine from gestation until post-natal week 8, we find no ^{15}N label retention by dividing small intestinal crypt cells after a four-week chase."



Experiments of DNA labeling of mature stem cells:

When labeling is initiated in the adult mouse after stem cell formation, older unlabeled strands serve as the template for new labeled strands. If stem cells have preserved a complement of template strands by biased segregation the template strands remain unlabeled and the complementary strands are obligatorily replaced with each round of division. Thus, a stem cell exhibiting biased segregation produces a daughter cell in which label is completely lost after the first division during a label-free chase. In contrast, cells exhibiting random DNA strand segregation equally distribute labeled strands to both progeny resulting in the 50% dilution of label with each division.

→ "In adult mice administered ^{15}N-thymidine pulse-chase, we find that proliferating crypt cells dilute the ^{15}N label, consistent with random strand segregation."



The following two pages show two of the several experiments described in the article referenced below.

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

Stem cell division and the immortal strand hypothesis (2/3)

Approximate halving of the ^{15}N-thymidine label between each group, compatible with 2 cycles of division. MIMS can follow the cell division cycle.

Mouse small intestine crypt cells: in-vivo methodology tests.

Thymidine is a pyrimidine deoxynucleoside. Deoxythymidine is the DNA nucleoside T, which pairs with deoxyadenosine (A) in double-stranded DNA.

Proliferating small intestinal crypt cells after continuous subcutaneous delivery for 1 week by surgically implanted osmotic minipump. Rapidly renewing small intestinal epithelial cells show dose-dependent nuclear incorporation of ^{15}N-thymidine after continuous administration.

Mouse small intestine crypt cells, in-vivo: cell division quantitation by the dilution of ^{15}N-thymidine label in nuclei during label-free chase.

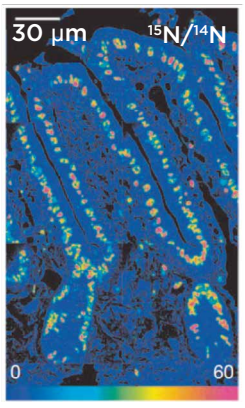
Mice pulsed 4 h with ^{15}N-thymidine (hourly intraperitoneal injections, 125µg), followed by 24-hour chase.

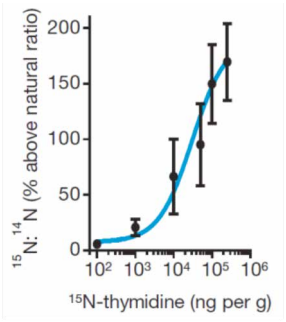
Cells segregate into 3 discrete groups of nuclear labeling:
- High
- Medium
- Lowest label.

Cells with same degree of labeling tend to cluster together.

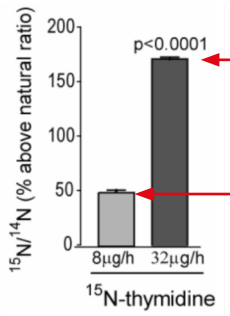
Highly labeled cells ("High") found at the leading edge of epithelial migration out of the crypt; Least labeled cells ("Low") remaining in the crypt.

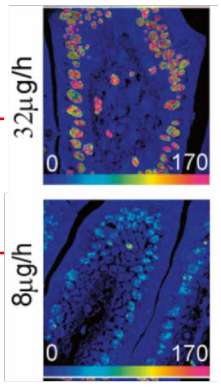
Small intestine. ^{15}N-thymidine labeling (one week) of nuclei extends from the crypts to the tips of the villi. Mosaic: 8 tiles, 80 µm each.



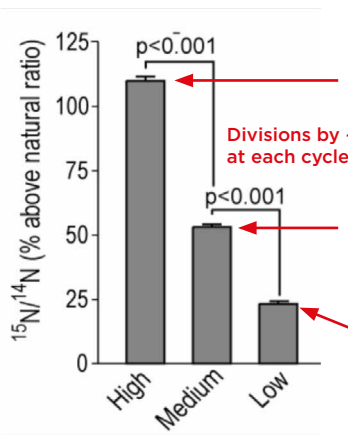


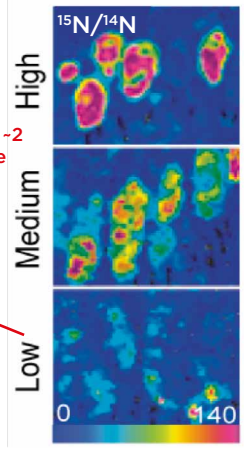
Dose response curve: ^{15}N-thymidine labeling after single sub-cutaneous injection (R2 = 0.99).





Data derived from the mean of all intranuclear pixels





Nuclear ^{15}N-thymidine *Crypt cell nuclei*

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

Stem cell division and the immortal strand hypothesis (3/3)

Stable isotope experiments coupled with NanoSIMS measurements demonstrate strong evidence for the invalidity of the "immortal strand" hypothesis

Adult mice intestine crypt cells: Label-dilution indicates random segregation of DNA strands. Bromodeoxyuridine (BrdU) is a synthetic nucleoside that is an analogue of thymidine. BrdU is commonly used in the detection of proliferating cells in living tissues. BrdU can be incorporated into the newly synthesized DNA of replicating cells (during the S phase of the cell cycle), substituting for thymidine during DNA replication.

Figure 1: PROTOCOL
^{15}N-thymidine administered for 2 weeks to adult mice (osmotic mini-pump, 20 mgh21), then BrdU for 24 h before euthanasia (See Supplementary Fig. 8).

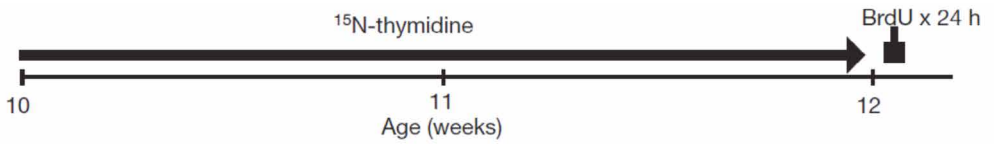


Figure 2:
Dividing cells (BrdU1) dilute 15N-thymidine label (small arrows) relative to undivided cells (BrdU2) (large arrows). Note two CBC cells with elongated nuclei at the crypt base.

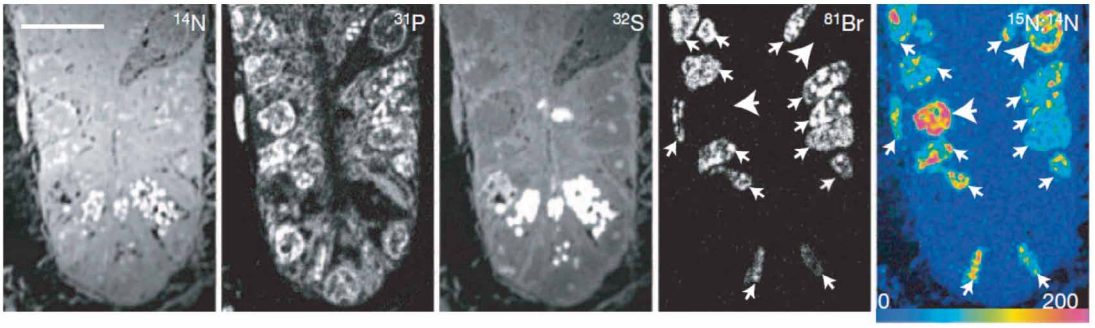


Figure 3:
Divided crypt cells (BrdU1), residing in CBC or +4-10 positions, demonstrated ^{15}N dilution consistent with one or two rounds of division during the chase (n53 mice per group).

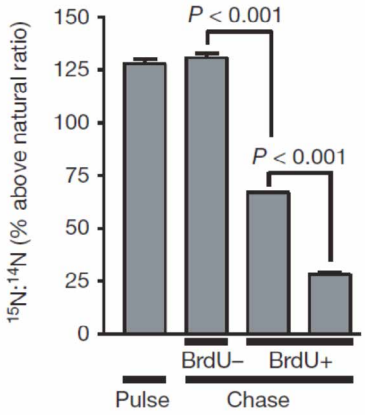
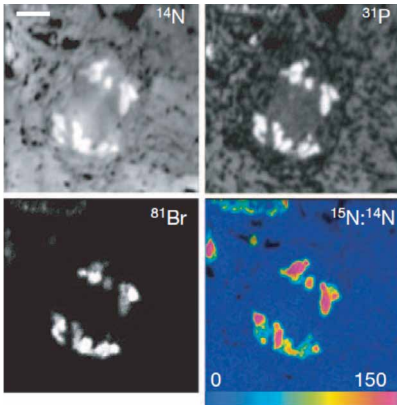


Figure 4:
Mitotic crypt cell
Segregating chromosomes are visible in ^{14}N and ^{31}P images. ^{15}N-label and BrdU were measured in *both* segregating chromosomal complements consistent with symmetric chromosomal segregation.

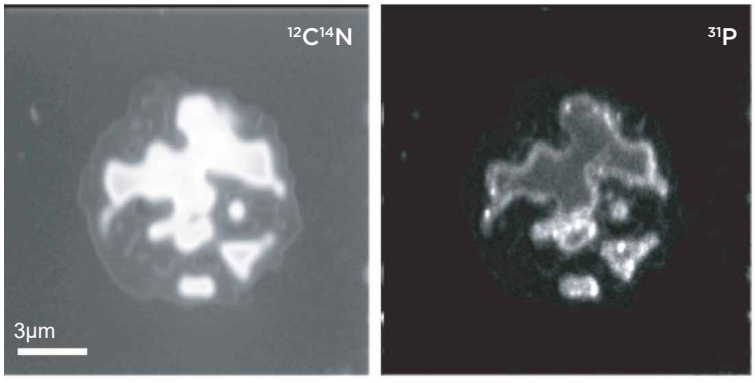


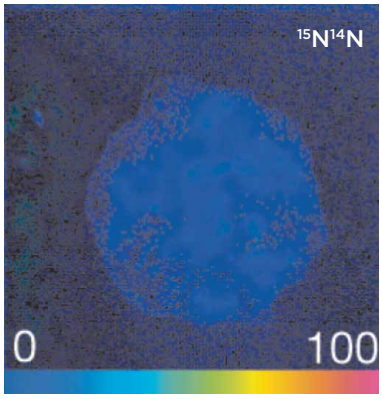
From: Multi-isotope imaging mass spectrometry quantifies stem cell division and metabolism. M. L. Steinhauser et al. , NATURE, Vol 481, 26 January 2012

Extending MIMS to metabolism and cell tracking in humans

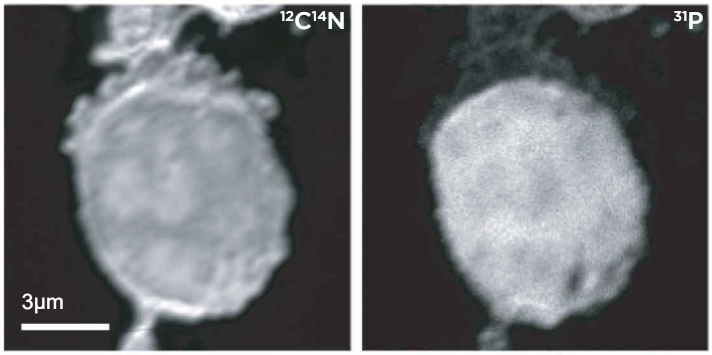
The NanoSIMS opens new perspectives in the detection and identification of individual labeled cells and their fate after transplantation or division.

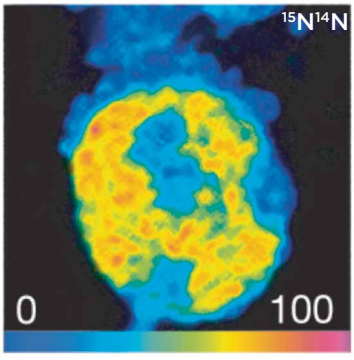
^{15}N-thymidine administered for 48 h to a healthy volunteer by intravenous infusion: no labeled white blood cell were found at the end of infusion (3600 analyzed). After a 4-week chase we found few labeled lymphocytes (2000 analyzed), consistent with a lag time before release from the bone marrow. There is no known risk of using stable isotopes.





Unlabeled polymorphonuclear leukocyte (PMN) with multi-lobed nucleus.

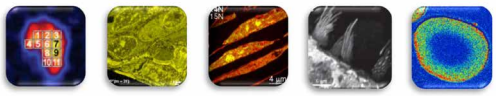




^{15}N-labelled lymphocyte after 4-week chase. The round nucleus seen in the ^{31}P image occupies the majority of the cell, as is t*ypical for lymphocytes.*

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

NanoSIMS 50L



A revolution in cellular biology



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