Understanding Aging & Cell Regeneration:

Cell turnover in Heart, Pancreas, Liver & Central Nervous System

Cell turnover measurements at the subcellular scale with NanoSIMS improve our understanding of aging, cell regeneration and self-renewal of organisms.

The NanoSIMS ion microprobe offers high lateral resolution imaging capabilities (50 nm) which enable the detection and quantification of isotopically labeled biomolecules within cells, organs, or tissues. Biomolecules are labeled with rare and stable D, ^{13}C,^{15}N, or ^{18}O isotopes. Their uptake in cells is measured by the NanoSIMS as an isotopic enrichment. Contrary to radioactive or halogenated labeling techniques, metabolic processes are not affected by isotopic enrichment.

Cardiomyocyte regeneration

Senyo et al., 2013, measured cardiomyocyte regeneration in normal growth and after triggering myocardial infraction using NanoSIMS at Institut Curie, France and at the National Resource for Imaging Mass Spectrometry, Harvard Medical School, USA. Previous studies had revealed that heart cells are generated in adult mammals, but the frequency of generation and the source of new heart cells had remained unknown.

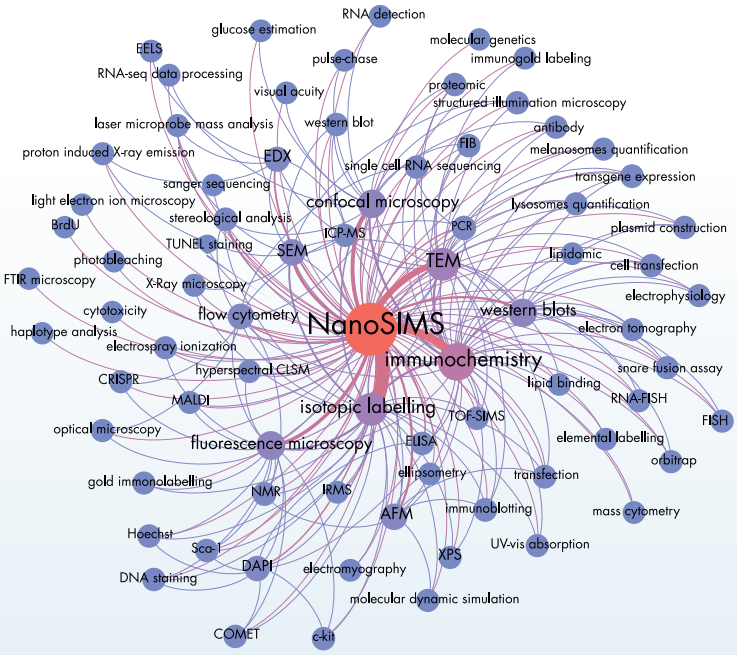
Methods:

^{15}N-labeled thymidine was injected for 8 weeks to three age groups of C57BL6 mice starting at day 4 (newborn), at 10 weeks (young adult) and at 22 months (old adult). NanoSIMS identifies the ^{15}N-rich nucleus of the cell.

Discoveries:

- NanoSIMS images of a cardiomyocyte section (Fig. 1) provide an accurate count of newly formed cells (white arrow, $^{15}N/^{14}N$~150 revealing the ^{15}N-labeled thymidine administered for 8 weeks to a young mouse) and of those present at birth (unlabeled, $^{15}N/^{14}N$~0). The proportion of newly formed cells over cells present at birth declines rapidly from the newborn (37% per year), to the young adult (6%) and to old mice (1%).
- Myocardial infraction seems to enhance the cardiomyocyte generation frequency (^{15}N rich) at 23% per year compared to unoperated mice with a cardiomyocite generation frequency of 4.4% per year.

If you are using one of the techniques below, you can no longer ignore NanoSIMS!



Network of techniques used in correlation with NanoSIMS in publications. Links are proportional to the number of citations.

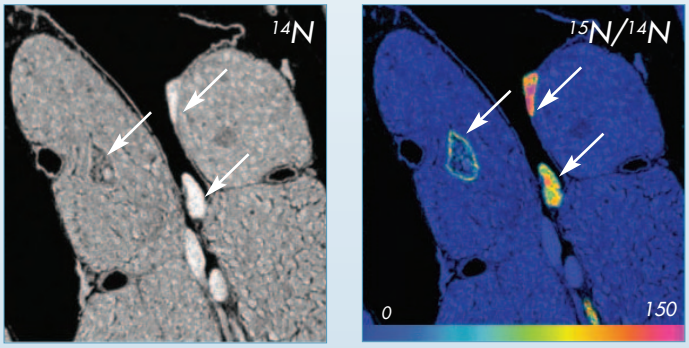


Figure 1: NanoSIMS imaging of cardiomyocite section. FOV 50 µm.

From: ***Senyo et al., Mammalian heart renewal by pre-existing cardiomyocytes, Nature, 493, 433–436 (2013).***

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Age mosaic in mouse organs

Arrojo e Drigo et al. (2019) measured the age of cells in liver, pancreas and the central nervous system using NanoSIMS at the Caltech Microanalysis Center, USA, at Institut Curie, France, and at the National Center of Excellence in Mass Spectrometry Imaging, NPL, UK. They observed diverse regeneration rates in different parts of an organ.

Methods:

Mice were initially fed with a pure ^{15}N diet from their embryonic stage until the 21st or 45th day of post natal development. Then, they were fed with chased ^{14}N-rich diet from 6, 18 and 26 months of age. NanoSIMS images identify new cells which are ^{14}N rich as opposite to the ^{15}N-rich cells that formed between the embryonic stage and the start of the ^{14}N-rich diet.

Discoveries:

- Pancreas islets consist of cells with wide range of ages: presence of old ^{15}N-rich cells (orange to red color) and ^{14}N rich young cells (blue). Most Alpha and Beta cells are as old as neurons. (Fig. 2).
- The central nervous system consists of cortical neurons, endothelial cell oligodendrocytes and perivascular fibroblasts with $^{15}N/^{14}N$ ratios 20-fold higher than natural $^{15}N/^{14}N$ after the 26th month chase. The brain cells are thus present since birth, confirming their life-long persistence. A single occurrence of a ^{14}N rich capillary endothelial cell shows that turnover can happen in the brain, but it is a rare event.
- In the liver, hepatocytes can be as old as neurons, but a small fraction still appears after 6 and 18 months. Sinusoid endothelial cells and stellate –like cells undergo turnover after 6 and 18 months.

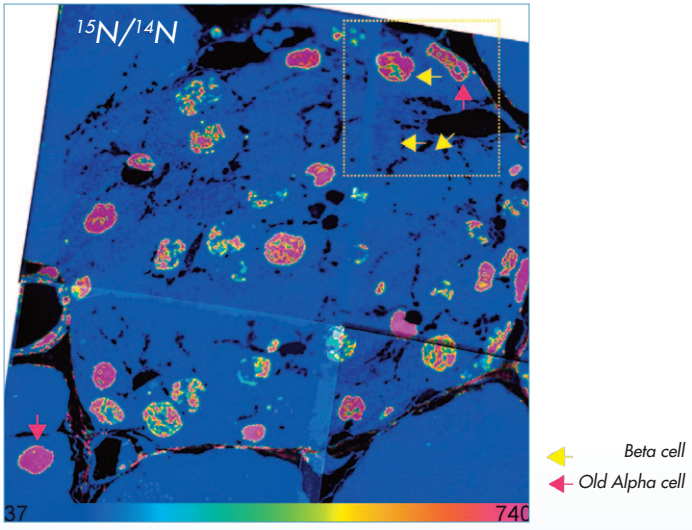

Figure 2: NanoSIMS imaging of islets of Langerhans in a pancreas section. FOV 70 µm.

From: ***Arrojo e Drigo et al., Age mosaicism across multiple scales in adult tissues, Cell Metabolism 30-2, 343-351 (2019).***

NanoSIMS: a revolution in cell biology

The NanoSIMS ion microprobe is uniquely capable of imaging and quantifying seven isotopes or elements in parallel (Fig. 3) over an area of up to several mm² and with 50 nm spatial resolution. Imaging techniques such as SEM, TEM, and fluorescence or confocal microscopy are often used to correlate structural information with isotopic or elemental content revealed by the NanoSIMS. The NanoSIMS measures the cell metabolism activity, lipid uptake and endocytosis of small labelled molecules, opening new horizons in biomedecine.

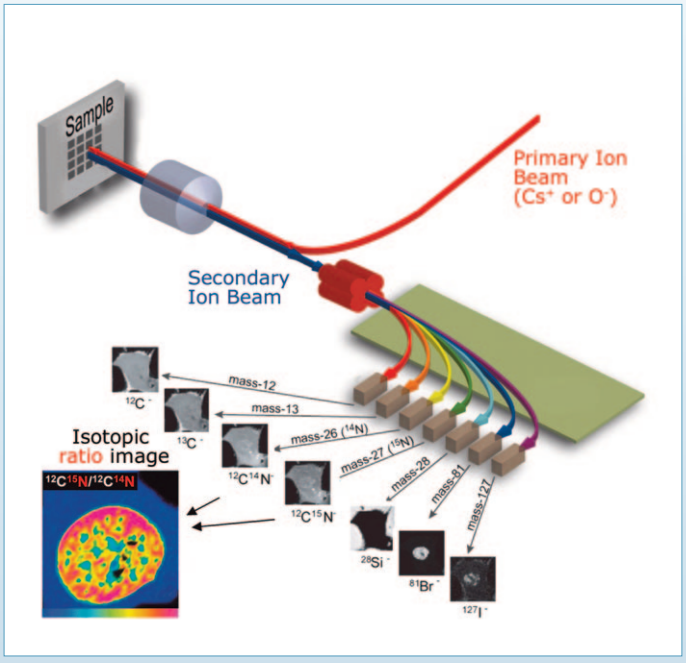

Figure 3: Synoptic of the NanoSIMS 50L secondary ion mass spectrometer.

From: From Steinhauser et al., Multi-isotope imaging mass spectrometry quantifies stem cell division and metabolism, Nature 481, 516–519 (2012).

Ref. #NS-22_01. May 2022