Developments on the NanoSIMS Ion Microprobe

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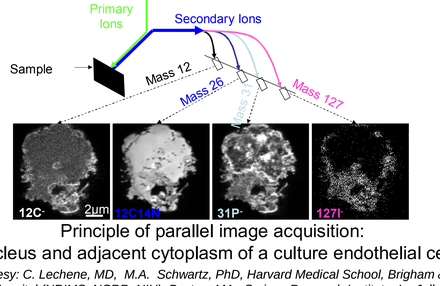
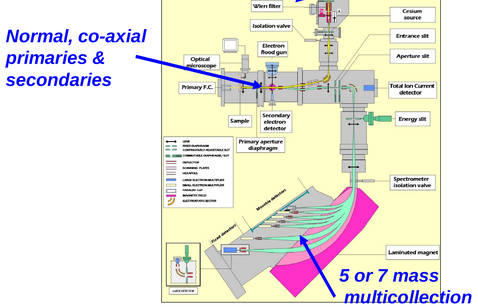
1) Description of the CAMECA NanoSIMS instrument.

Similar to a scanning electron microscope (SEM), a beam of Cs^+ or O^- primary ions is focused and rastered on the surface of the sample, down to **50nm beam size (= spatial resolution).** The ions sputtered by this primary beam are collected, and this 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 species or isotopes can be simultaneously recorded, originating from the exact same sputtered volume, ensuring reliable isotopic ratio and perfect image registration. [1]



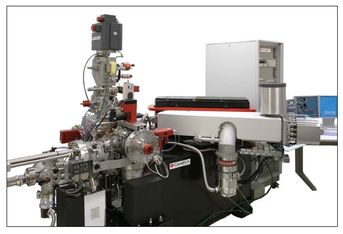
Principle of parallel image acquisition:
Nucleus and adjacent cytoplasm of a culture endothelial cell

Courtesy: C. Lechene, MD, M.A. Schwartz, PhD, Harvard Medical School, Brigham & Women's Hospital (NRIMS, NCRR, NIH), Boston, MA., Scripps Research Institute, La Jolla, CA, USA.



Synoptic of the NanoSIMS

NanoSIMS 50 (5 masses)



NanoSIMS 50L (7 masses, EMs + FCs)

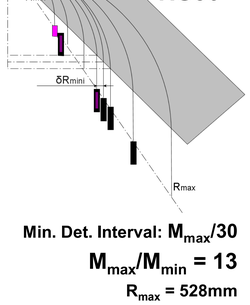
2) Two versions: NS50 and NS50L

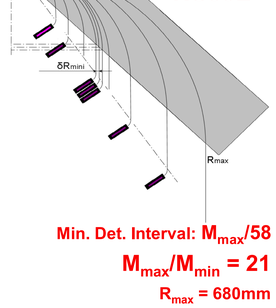
Standard NS50 multicollection

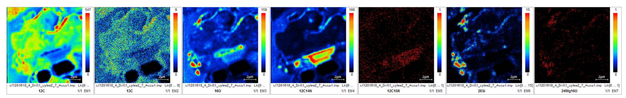
Minimum mass interval:
On the standard NS50 magnetic sector analyzer, the width of the E.M. detector (8mm) and the geometry (angle between focus line and incoming ion) limit the minimum mass interval of two neighbor detector to M/30: for example 28, 29, 30amu can be measured simultaneously but only 87, 90, 93 can be measured in parallel and 87, 88, 89 must be measured in three magnetic field steps.
More exactly, δM_{mini} (mini mass interval between 2 adjacent det.) = sqrt (M_{max}) * sqrt (M) * δR_{mini}/ 264. or: $\mathbf{\delta M_{min} \sim sqrt\ (M_{max}) * sqrt\ (M) * 0.032.}$

NS50L multicollection

Examples of new possibilities for parallel acquisition, not possible with the NS50,:
Materials: 40Ca, 48Ti, 51V, 52Cr, 55Mn, 56Fe, 58Ni.
Cosmochemistry: 12C/16O/17O/18O/28Si/29Si/30Si, or 32S/33S/34S, or 40Ca/44Ca/46Ti/47Ti/48Ti/49Ti.
δM_{mini} (mini mass interval between 2 adjacent det.) = sqrt (M_{max}) * sqrt (M) * δR_{mini}/ 340. or: $\mathbf{\delta M_{min} \sim sqrt\ (M_{max} * M) * 0.017.}$





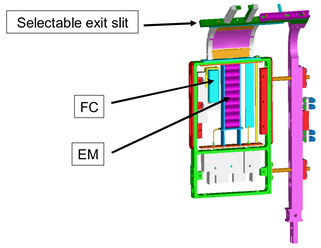


NS50L: Seven masses recorded in parallel. Extra-terrestrial material, FoV:12 X 12 µm. Image by courtesy of S. Messenger, NASA-JSC, USA

3) E.M/ FC switch on the NS50L

The Electron Multipliers (E.M) detect each single ion, covering a range 0 to a few 1E6 counts per seconds. In order to be able to:
- measure larger signal, specially for intense mono-isotopic species,
- suppress EM limitations: aging, noise, variation of yield on the first dynode, loss of ions detected simultaneously (QSA),…
- improve precision, reproducibility and detector inter-calibration,
the trolley assembly has been redesigned to allow **simultaneous equipment of each trolley with one Electron Multiplier (EM) and one Faraday Cup (FC).**

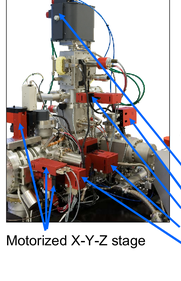
The two detectors can be mechanically commuted behind the exit electrostatic sector, the multicollection being opened at atmospheric pressure. The NS50L can be equipped with seven E.M and seven FCs. The noise of the FC is 5E-16 A over 5sec., equivalent to a noise of 3125c/s.
The Multiple FCs coupled with the use of nA primary current/ µm primary beam size opens the door to isotopic measurements on the NanoSIMS with sub-permil reproducibility for Geochemistry applications.

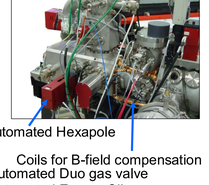


NS50L detector trolley
allowing FC / EM selection
after exit slit and cylindrical sector

4) New Software and Automation:

- **Aperture and Slits:** computer control of all slits (entrance, aperture, energy, exit), apertures (source, coaxial lens) and hexapole centering.
- **Sample stage:** third axis (Z) automated as X and Y.
- **Ion Source:** the duoplasmatron gas valve is automated
- **High Reproducibility:** Residual B-field compensation with Helmoltz coils and automated beam centering through the secondary ion path.





- Stage Raster: although the NanoSIMS is devoted to small area analysis (typically < 50µm), **FoV of several mm wide** can now be imaged in the Stage Rastering mode (combined with beam scanning to make use of all pixel area) in order to avoid mosaic images with not-always-perfect joins. The result file is a *.im standard image. Available.

- Point Logger: a photo of a sample or sample holder is recorded with an external tool (electron or optical microscope, camera, scanner,..) and imported in the NS50 software. This image must include at least two reference points. The sample is then loaded in the NanoSIMS. Re-alignement is calculated after validationg of the two reference points in SIMS position. From then on, navigation can be performed directly on the imported image: the NS50 stage will send the stage to any position selected in the imported image field with a few microns/ tens micron precision (depending on positions). Available.

- Ultra Low Energy pre-cleaning/implantation: the NS50L works at high impact energy (16keV). In order to reduce the transient depth and avoid wasting materials during the implantation of reactive species (O or Cs) in precious or ultra-thin surface layers, a Ultra Low Energy (few hundreds eV) implantation (decelerating) mode has been developed. Basically the sample is polarized very near the potential of the ion source. Validation tests started. Succesful implantation in silicon at 100eV impact energy.

- New PC-Windows Image processing software: WinImage. Based on Aphelion extended scientific Image processing library already used in the EPMA SX100. The new software allows image drift registration, flexible ROI analysis (ratio, line-scan, depth profile), various Process functions (scan Accumulation, Normalization, Isotope Deviation Map, Linear Combination, 3D display) and extended copy-paste-print functions. Available.

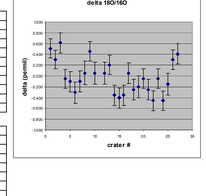
5) Other on-going or scheduled developments:

- **Automated in-situ FC-EM switch** for the NS50L. Planned availability: 2009.
- **Larger max. primary current** for spots of a few µm: from presently 2-4nA up to **10-20nA** in the next design of primary column. In addition, better positioning of the octopole for a better stigmatism adjustment at high primary current. Planned availability: 2009.
- Feasibility test of **cold stage** for biological samples. Parts designed, under manufacturing-assembly. Tests in Q3-Q4 2008. The idea (hope) is to be able to by-pass sample preparation in biology (i.e. plant cells with large vacuoles) and work on frozen-hydrated sections.
- Integration of **automated particle analysis** routine. 2008.
- Characterization of **anti-scattering blades** in NS50 magnet tube: similar to the NS50L and IMS 1280 the multicollection chamber of the NS50 now incorporates blades aiming at reducing scattering of secondary ions inside the multicollection wall when using the NS50 with several nA primary current. Planned Q3-Q4 2008.
- Complete **Switch from Unix to PC-Windows**: final tests scheduled in Q3-4 2008.

6) Oxygen isotope Reproducibility on quartz (FC-FC mode)

(acceptance tests for MPI Bremen, feb 2008)

10x10 microns	Cs+ 16keV, 3-4 nA	100s	18O=0,5 pA	
1rst crater series: 10 craters of 10X10 µm spaced by 30µm				
Time (mn)	18O/16O	Err Mean	18O/16O	Err Mean
2	1.9633E-03	1.77E-02	0.510	1.77E-01
6	1.9629E-03	1.72E-02	0.306	1.72E-01
10	1.9635E-03	1.80E-02	0.612	1.80E-01
14	1.9622E-03	1.77E-02	-0.051	1.77E-01
18	1.9621E-03	1.87E-02	-0.102	1.87E-01
22	1.9617E-03	1.95E-02	-0.306	1.95E-01
26	1.9621E-03	1.92E-02	-0.102	1.92E-01
30	1.9624E-03	2.17E-02	0.051	2.17E-01
34	1.9632E-03	1.81E-02	0.459	1.81E-01
38	1.9624E-03	1.99E-02	0.051	1.99E-01
2nd test: 16 craters (4 x 4) of 10X10 µm spaced by 2.6mm				
42	1.9624E-03	2.02E-02	0.051	2.02E-01
46	1.9627E-03	1.76E-02	0.204	1.76E-01
50	1.9618E-03	1.88E-02	-0.357	1.88E-01
54	1.9615E-03	1.86E-02	-0.408	1.86E-01
58	1.9616E-03	1.86E-02	-0.357	1.86E-01
62	1.9624E-03	1.95E-02	0.051	1.95E-01
66	1.9618E-03	1.90E-02	-0.255	1.90E-01
70	1.9619E-03	2.05E-02	-0.204	2.05E-01
74	1.9622E-03	1.79E-02	-0.051	1.79E-01
78	1.9618E-03	1.94E-02	-0.255	1.94E-01
82	1.9614E-03	1.88E-02	-0.459	1.88E-01
86	1.9622E-03	1.84E-02	-0.051	1.84E-01
90	1.9614E-03	1.77E-02	-0.459	1.77E-01
94	1.9620E-03	2.01E-02	-0.153	2.01E-01
98	1.9620E-03	1.84E-02	0.306	1.84E-01
102	1.9631E-03	1.84E-02	0.408	1.84E-01
Sigma (permil)	0.310			
Std-Err (permil)	0.061			

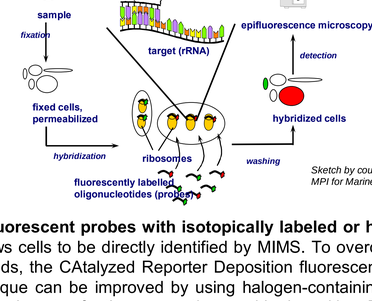


Working with nA probe current makes the use of multiple FC practical and demonstrates low sub-permil reproductibility.

7) A growing method in microbiology: FISH-SIMS

7A) FISH (Fluorescent In-Situ Hybridization)

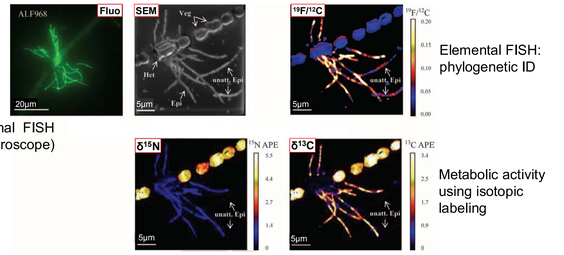
It is based on the use of fluorescent-labeled probes that are specific to the organism of interest and that bind to the intracellular 16S ribosomal RNA.



Sketch by courtesy of Dr. Marcel Kuypers MPI for Marine Microbiology, Bremen - Germany

Replacing fluorescent probes with isotopically labeled or halogenated probes (I, Br, F…) allows cells to be directly identified by MIMS. To overcome the natural F and Br backgrounds, the CAtalyzed Reporter Deposition fluorescence in-situ hybridization (FISH) technique can be improved by using halogen-containing fluorescently labeled tyramides as substrates for the enzymatic tyramide deposition. Ref [2]

7C) Exchanges of nitrogen and carbon in a dual-species microbial consortium



Microbial consortium consisting of filamentous cyanobacteria (*Anabaena* sp. strain SSM-00) and heterotrophic alphaproteobacteria (*Rhizobium* sp. strain WH2K) attached to heterocysts. Enhanced Element - FISH using an alphaproteobacteria probe (ALF968). Images taken after 24-h incubation with 13C-bicarbonate and $^{15}N_2$.
Het, heterocyst; Veg, vegetative cell; Epi, epibiont; unatt Epi, Epibiont cells not attached to heterocysts.
The Heterocysts fix nitrogen and export it to the vegetative cells and the *attached* epibiont. The **unattached epibionts**, although phylogenetically identified are not enriched in 13C nor in 15N.

Thus in one single measurement the NS50 allows to identify the bacteria (with EL-FISH), measure their metabolic activity and reveal their interactions. This method is applicable on unculturable cells that represent the majority of environmental bacteria.

From: Applied And Environmental Microbiology, May 2008, Sebastian Behrens et al.

8) Conclusion

A continuous NanoSIMS development program is going on.
The aims are a better isotopic reproducibility (large Ip, multiple FCs, coils, automation), a larger flexibility or range of applications (high primary current, Low energy pre-implantation, easier navigation) or specific application (particle analysis, cold stage).

REFERENCES
1. G. Slodzian, B. Daigne, F. Girard, F. Boust, High Sensitivity and High Spatial Resolution Ion Probe Instrument, Proceedings of the 6th SIMS Conference, Versailles sept. 87.
2. Pernthaler, A., Pernthaler, J. & Amann, R. (2004) in Molecular Microbial Ecology Manual, eds. Kowalchuk, G. A., de Bruijn, F. J., Head, I. M., Akkermans, A. D. L. & van Elsas, J. D. (Kluwer Academic Publishers, Dordrecht/Boston/London), pp. 711-726.