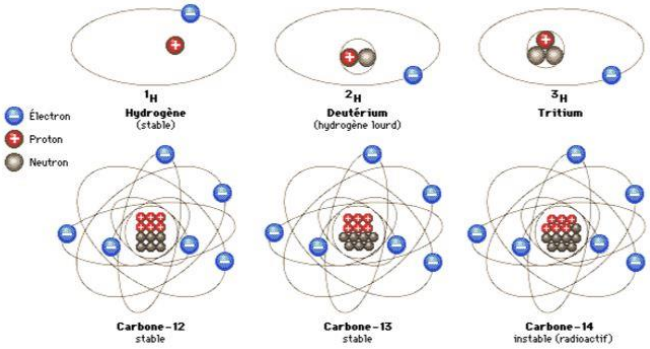
Imagerie isotopique : perspectives en biologie

Jean-Luc GUERQUIN-KERN

Institut Curie /INSERM U1196 (CMIB)

Laboratoire de Microscopie ionique / Plateforme PICT-IBiSA

Isotopes en biologie



Dès la découverte des isotopes il y a un siècle, leur utilisation en biologie a été envisagée avec le marquage de molécules par des isotopes lourds (G. Hevesy; R. Schoenheimer) qui se comportent presque comme des « molécules normales » mais qui peuvent être caractérisés par des critères physiques (masse, radioactivité)

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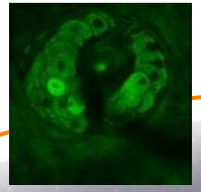
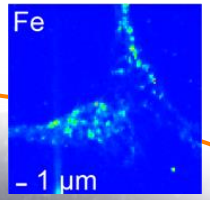
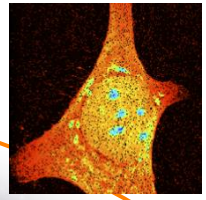
Chemical, Molecular, Elemental, Isotopic Imaging?



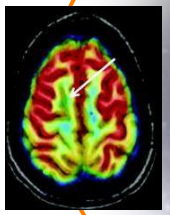
Vibrational imaging (IR, Raman)





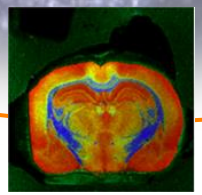
Elemental imaging (X-RSF synchrotron radiation, PIXE,

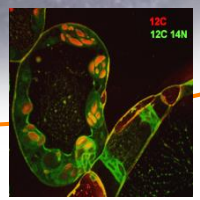
In vivo

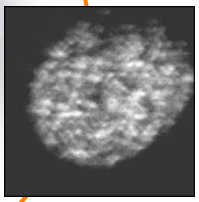


Molecular imaging TEP/IRM/optical imaging

Molecular , Elemental & isotopic imaging by MS (MALDI-TOF, LA-ICP, SIMS)







Elemental imaging by EFTEM/STEM

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SIMS Analysis

Principle and
Characteristics of ion analysis

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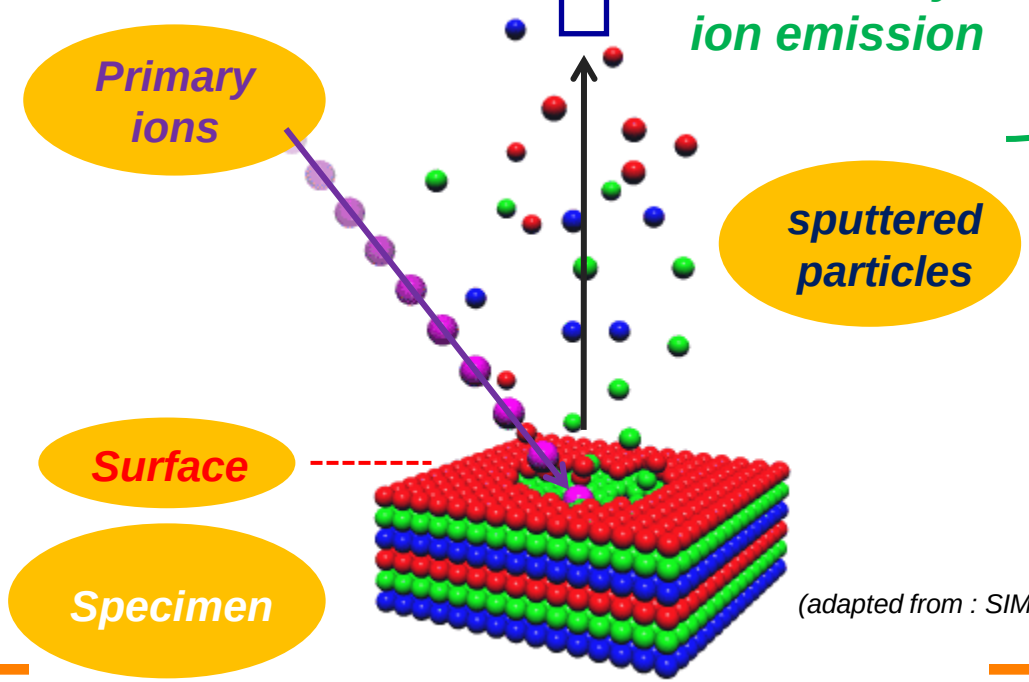
SIMS (Secondary Ion Mass Spectrometry)

Mass Spectrometer
separation according to mass/charge ratio (M/q)

Secondary ion emission

Chemical Analysis
(elemental and isotopic)



Neutral *(majority)*
Charged particles
electrons
secondary ions
positive ions
negative ions

(adapted from : SIMS workshop, http://www.simsworkshop.org)

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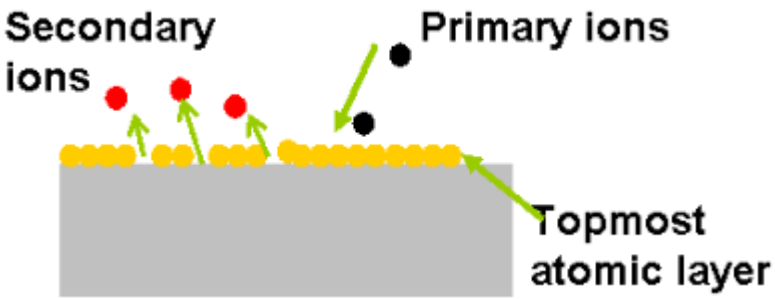
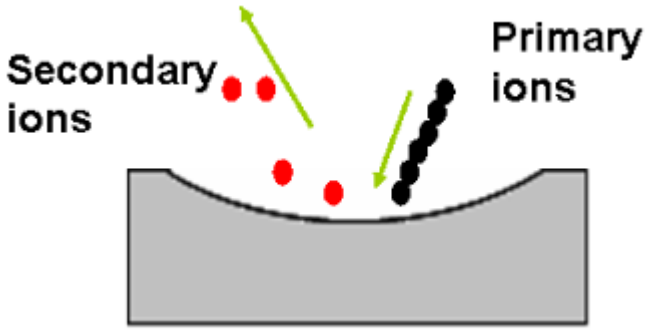
Operating modes

Static SIMS



- ✓ very low primary current density (<10^{12} ions/cm^2) : each subsequent primary ion hits an undamaged area
- ✓ chemical analysis of the uppermost layer of the sample

Dynamic SIMS



- ✓ high dose primary current density (>10^{17} ions/cm^2) : primary ion bombardment changes the structure of the area under analysis
- ✓ secondary signal comes from slightly deeper layers

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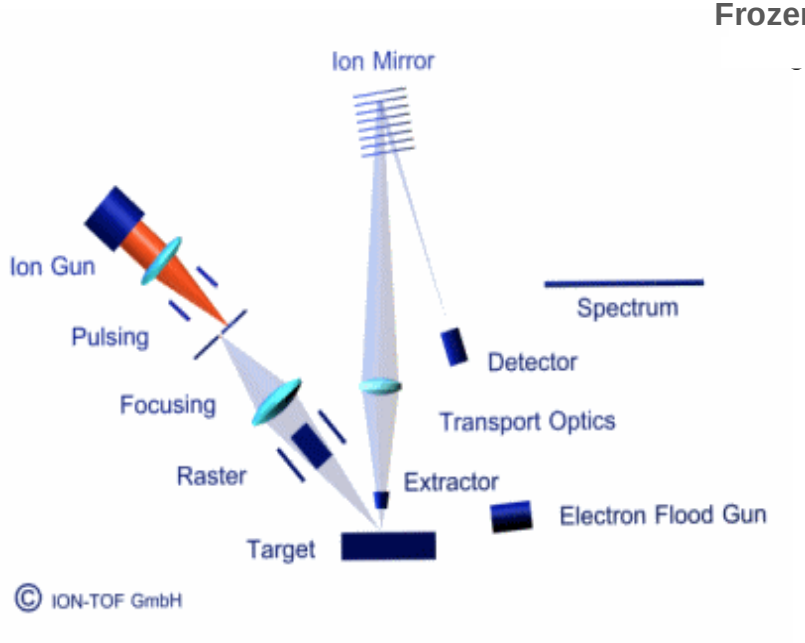
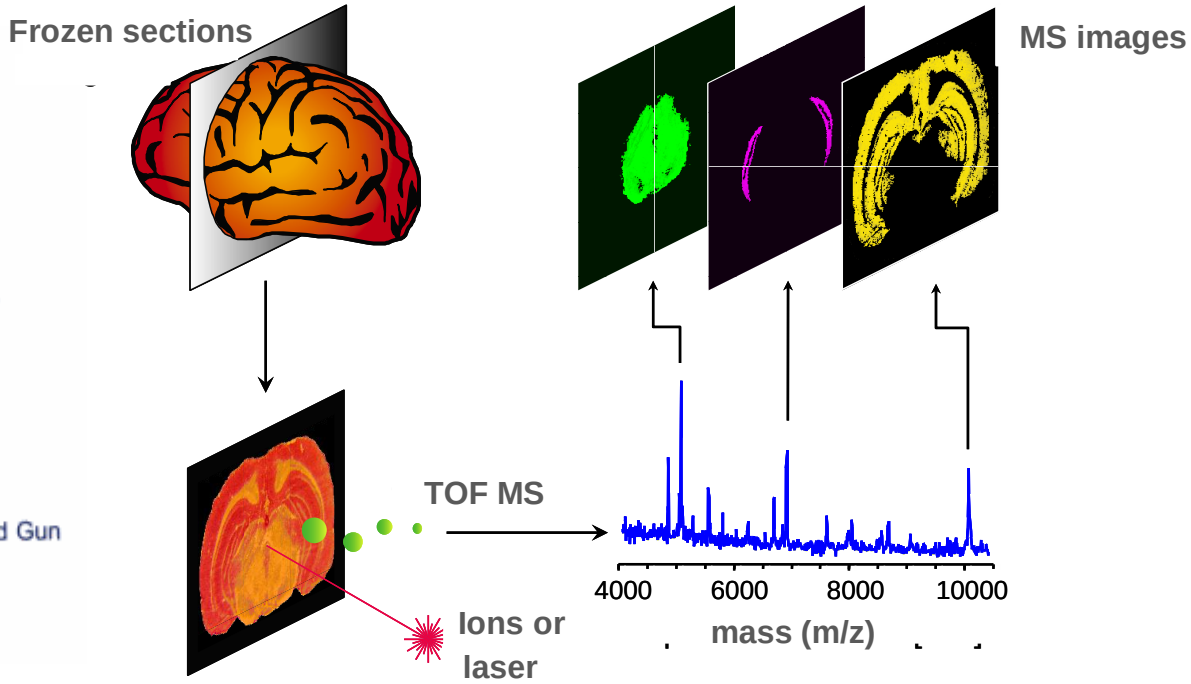
Static SIMS

TOF-SIMS (MALDI-TOF)





from Stoeckli M et al, Nature Medicine ,2001, 7, 493-496.

Low fragmentation = molecular imaging

complex spectra on a broad mass scale

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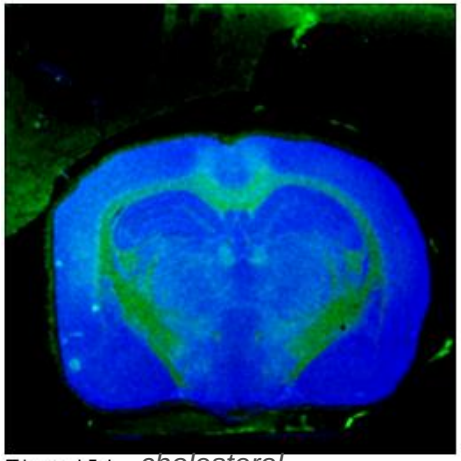
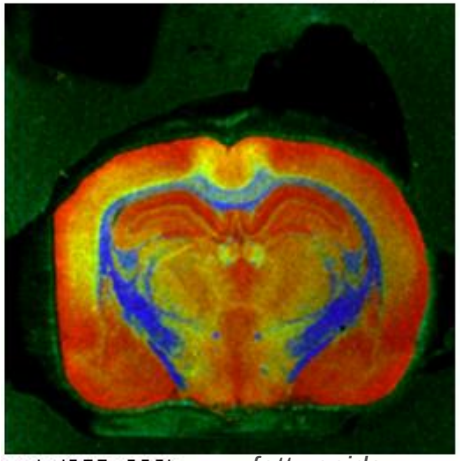
Static SIMS

TOF-SIMS with Bi_3^+ cluster ion source

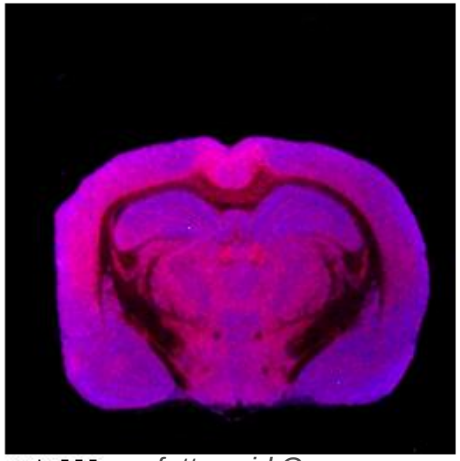
Field of view: 18000.0 x 18000.0 µm²



Blue=184 *cholesterol*
Green=769 *phospholipids*



red=(255+283) *fatty acids*
green=892 blue=771 *triglycer*



red=283 *fatty acid C_{16}*
blue=255 *fatty acid C_{16}*

256x256 pixels
pixel size: 70x70 µm2
acquisition time: 3h

ICSN Gif (O. Laprévote)

from Touboul D et al, J Am. Soc Mass Spectrom,2005, **16:** 1608-1618.

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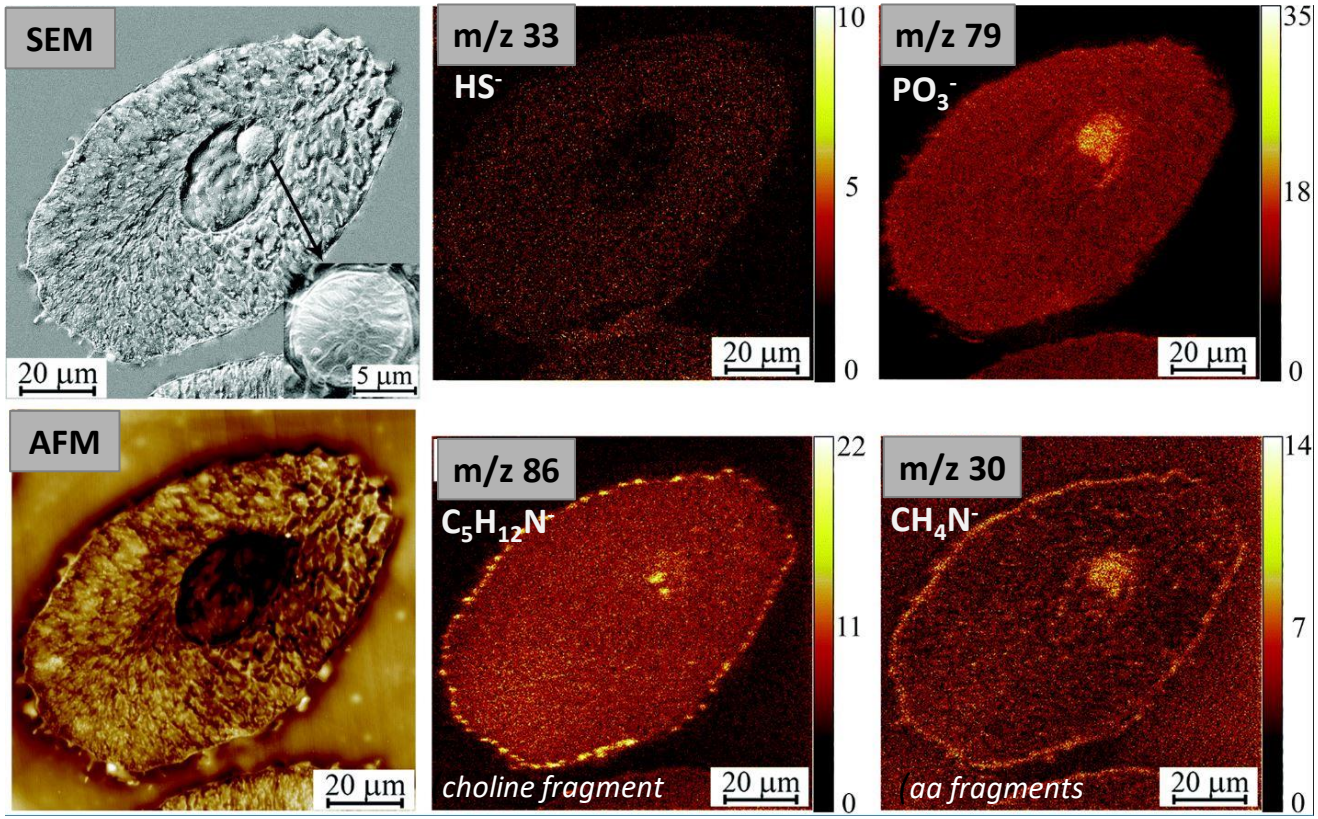
Static SIMS



Oocyte : section 2µm

from *Gulin A et al*, Analyst, 2014, **141:** 4121-4129

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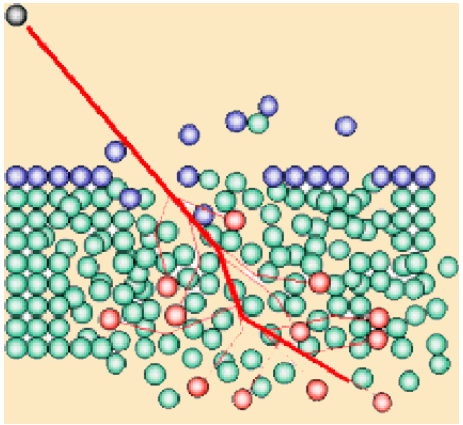
Dynamic SIMS

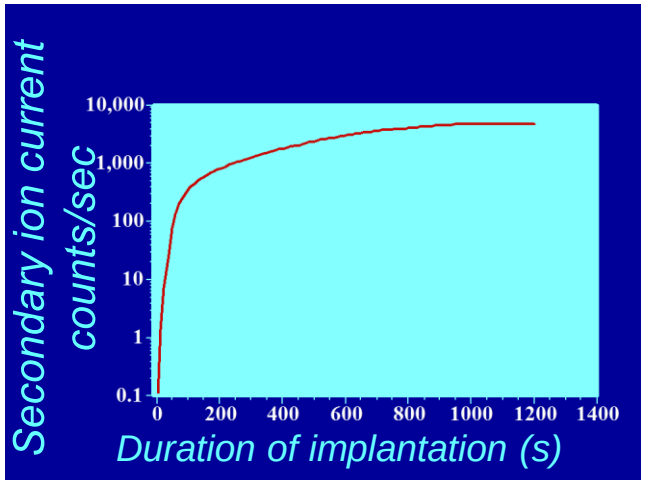


- cascade of collision created by the high primary current density

- secondary current intensity varies with primary ion implantation

- high fragmentation:

(↗ number of elements to analyze but loss of molecular informations)



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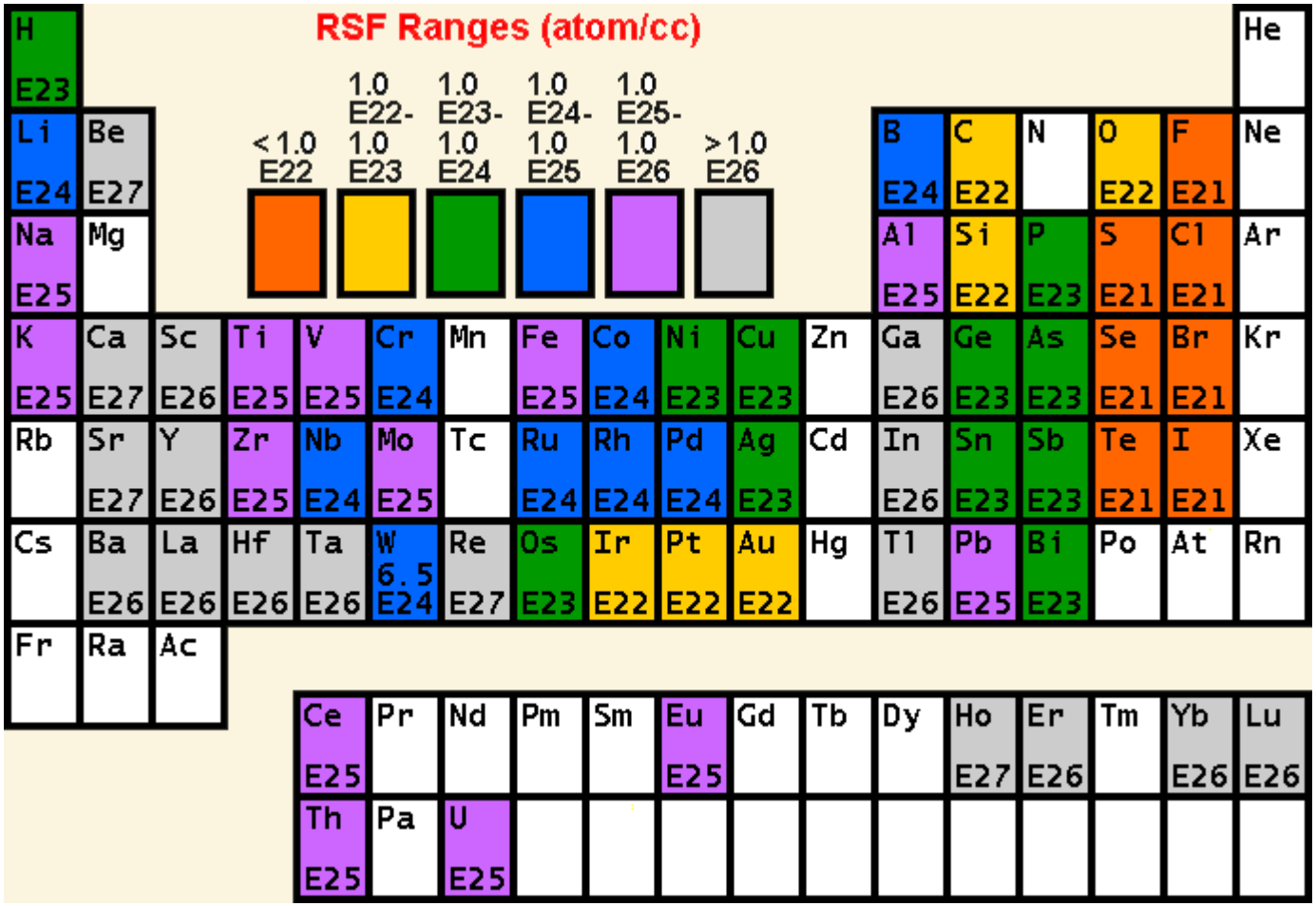
Relative Sensitivity Factor

(Cs bombardment on Si matrix)



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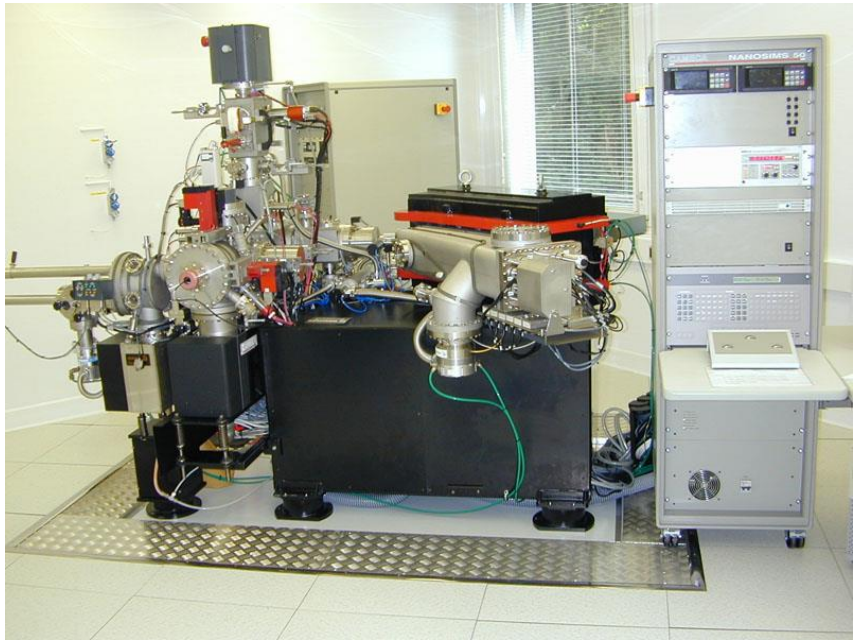
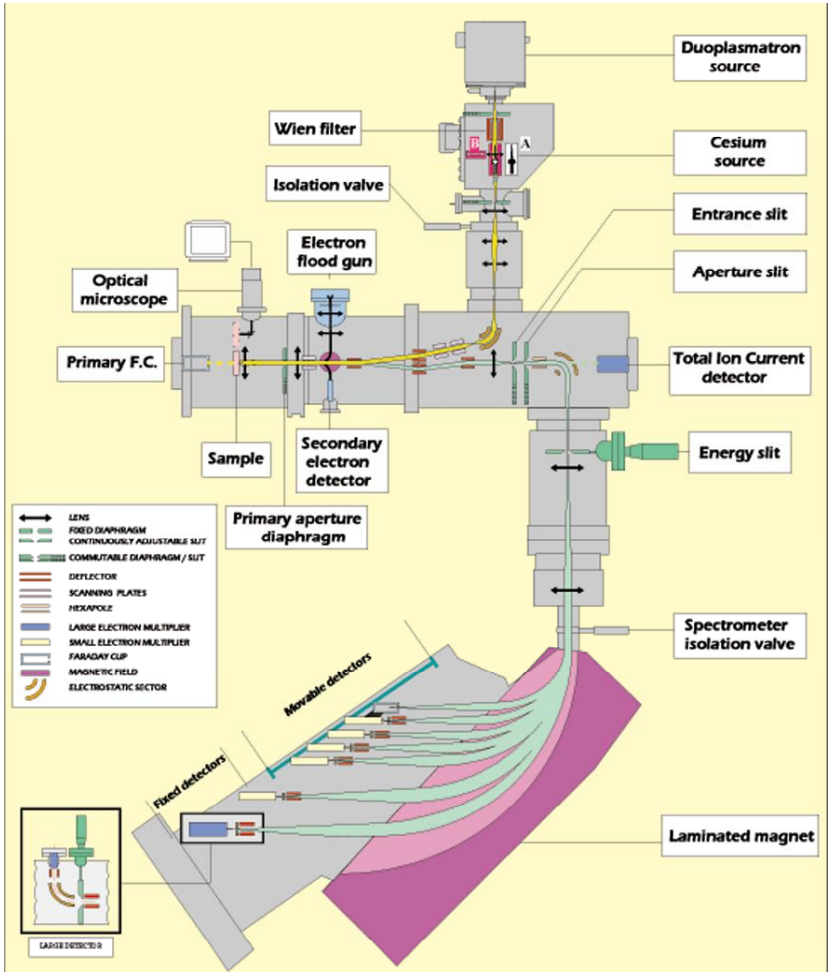
Characteristics of NanoSIMS





✓*Ion micro-probe with a high spatial resolution (≤50nm),*

✓*ability to measure simultaneously up to seven masses coming from the same microvolume*

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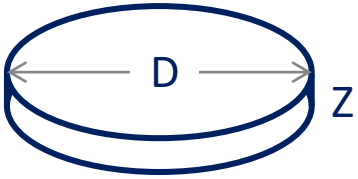
Spatial resolution: limit for analytical imaging



$N_A = 6{,}02 \times 10^{23}$

D	Z	volume (L)	1 mM
10 µm	1 nm	10^{-16}	60000
1 µm	0,1 nm	10^{-19}	60
100 nm	1 nm	10^{-20}	6

cell Vol ≈ 10^{-12} L

3 10^9 prot/cell ….

50 prot/ anal vol with the100nm probe : 2.5 10^5 atoms (6 10^4 C; 1.5 10^4 N; 10^3 S)

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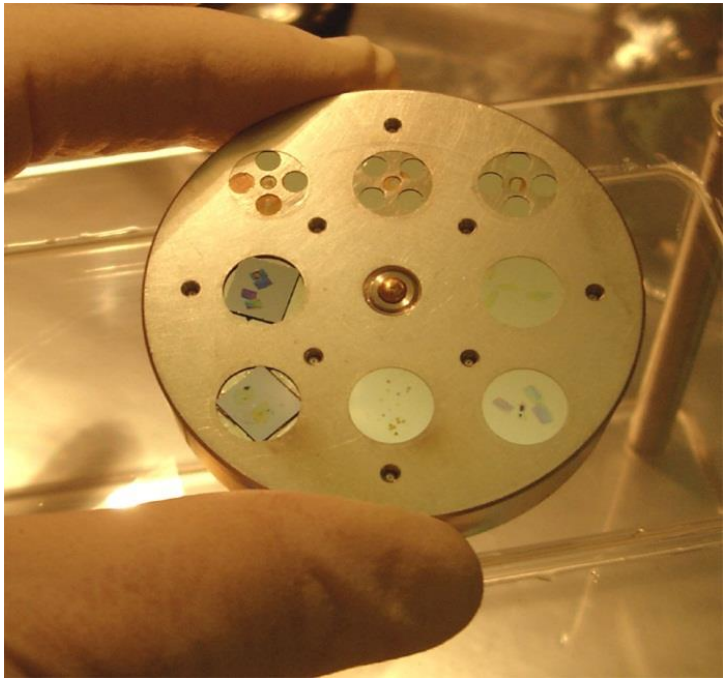
Sample characteristics

- ✓ Samples are analyzed at room temperature under ultra high vacuum
- ✓ Specimen should be fixed and dehydrated as for TEM analysis



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Application domains

“Pharmacology”

Elemental analysis

Isotopic analysis

- **metabolism**
- **cytometry**

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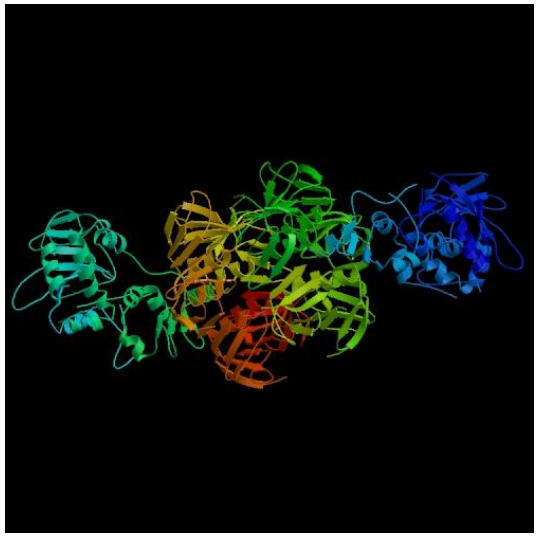
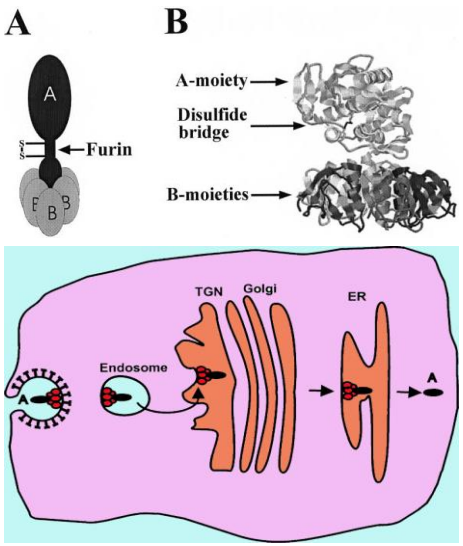
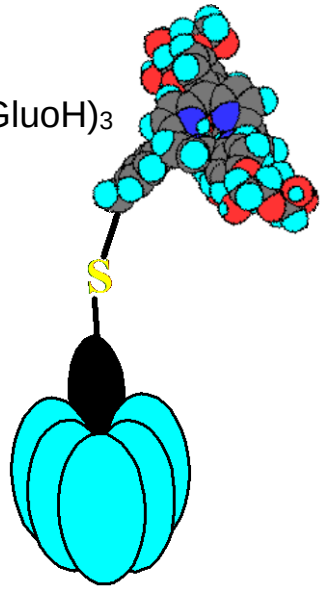
“Pharmacology”

W Römer, L. Johannes, UMR 144 CNRS/ Institut Curie Recherche

Drug targeting using B-subunit of Shiga toxin (STx-B)







B-subunit

^{15}N-recombinant protein

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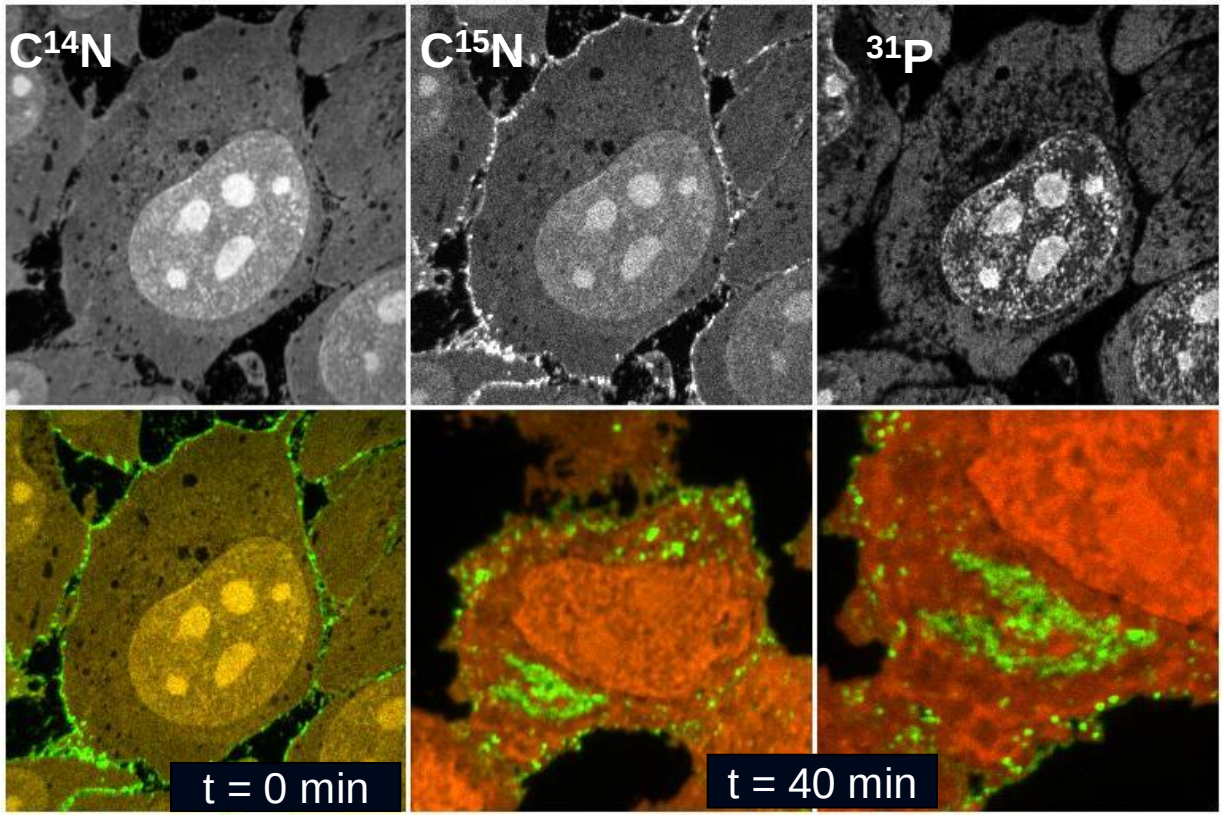
“Pharmacology”

sub-cellular distribution of ^{15}N-STxB



Field of view = 20 µm

Fov = 10 µm

*W. Roemer et al, Appl. Surf. Sci., (2006), **252**: 6925-6930*

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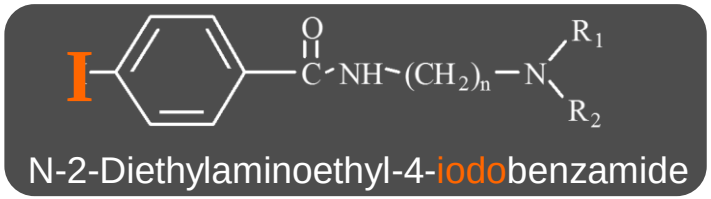
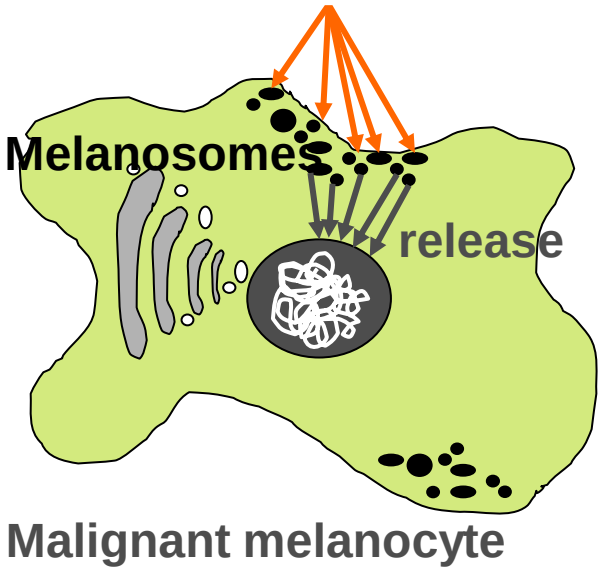
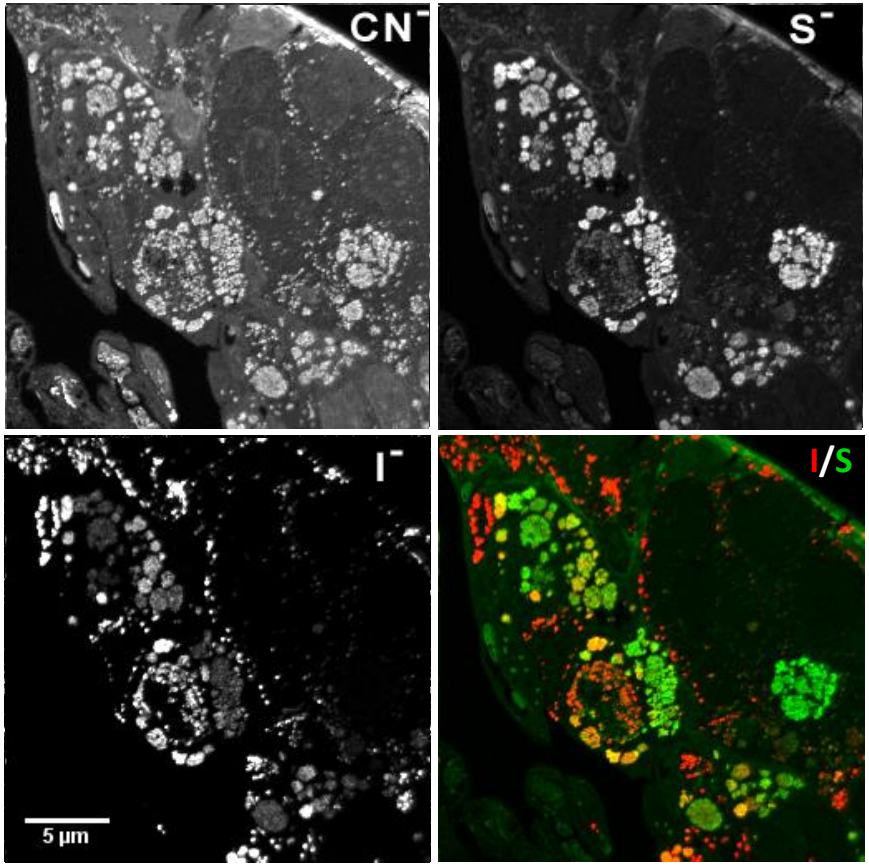
“Pharmacology”

I-BZA in a strategy of targeted-melanoma therapy

N Moins, INSERM U990, Clermont-Ferrand







*Bonnet-Duquennoy M et al, Int J Cancer, 2009, **125(3)**:708-716*

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Application domains

metabolism studies

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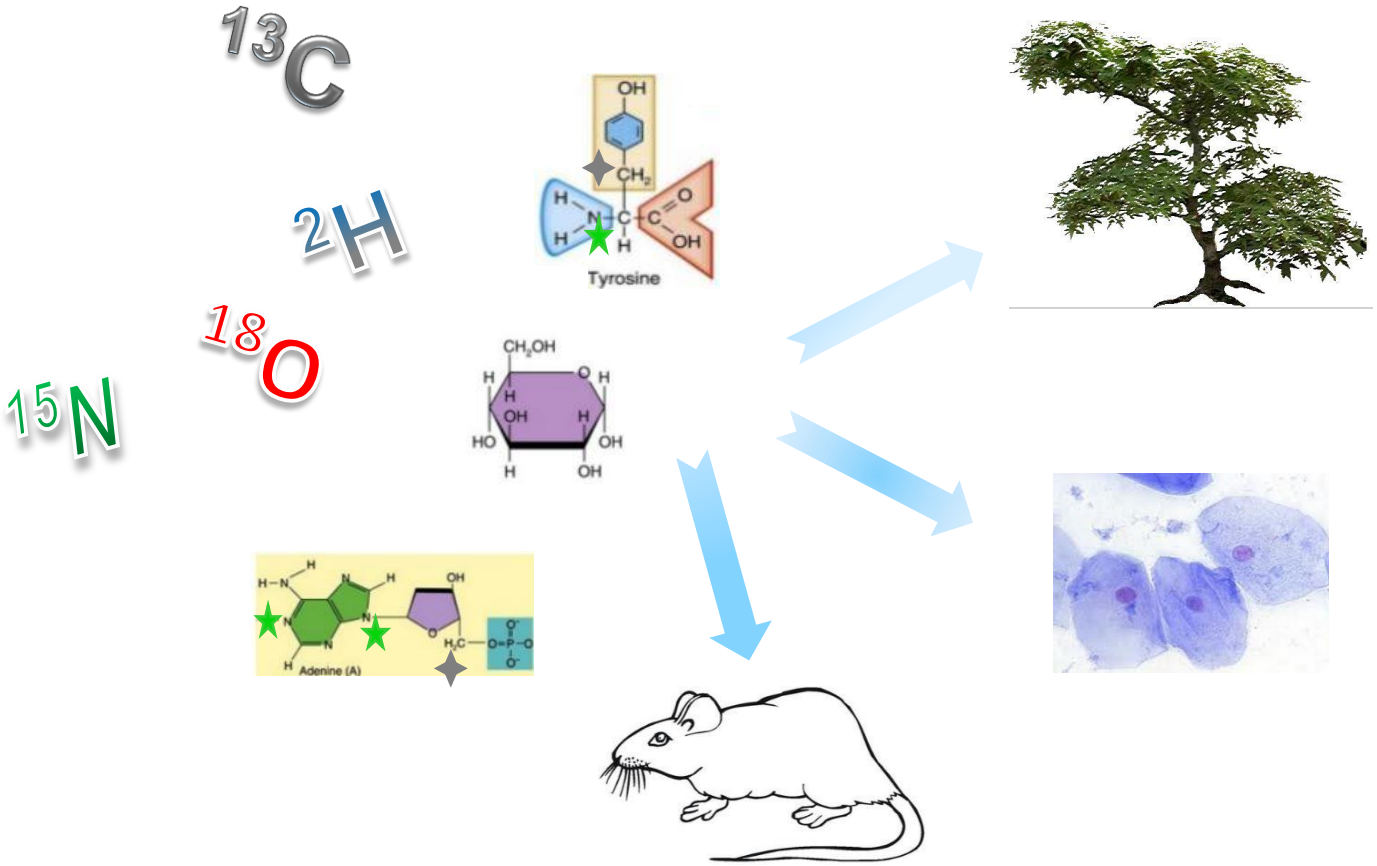
Isotopic labeling



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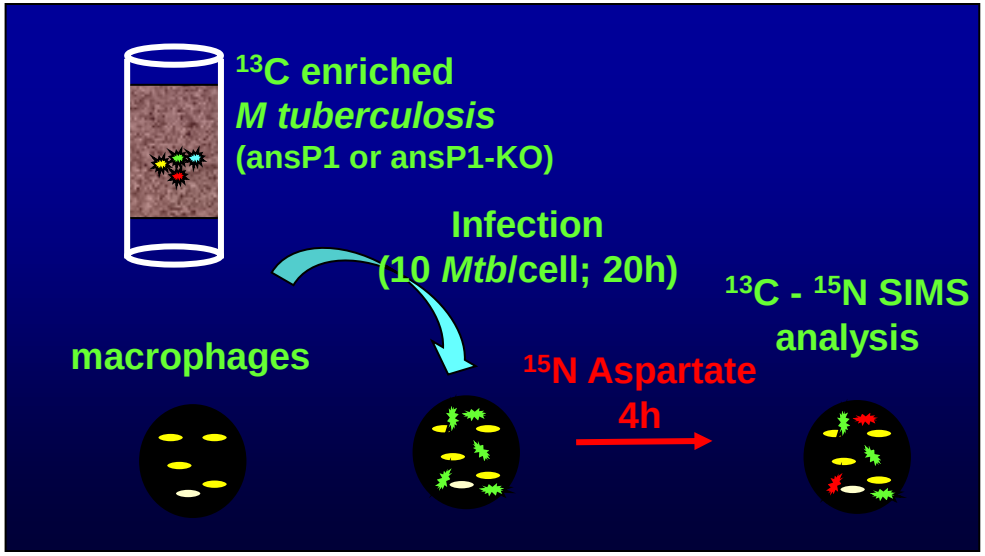
Metabolism studies :

Imaging of *Mycobacterium tuberculosis* nitrogen assimilation

Collab. O. Neyrolles, IPBS/CNRS, Toulouse

Inhibition of host-derived catabolic pathways as promising therapeutic approaches for tuberculosis

Hypothesis: AsnP1 as essential transporter in assimilation of nitrogen from aspartate



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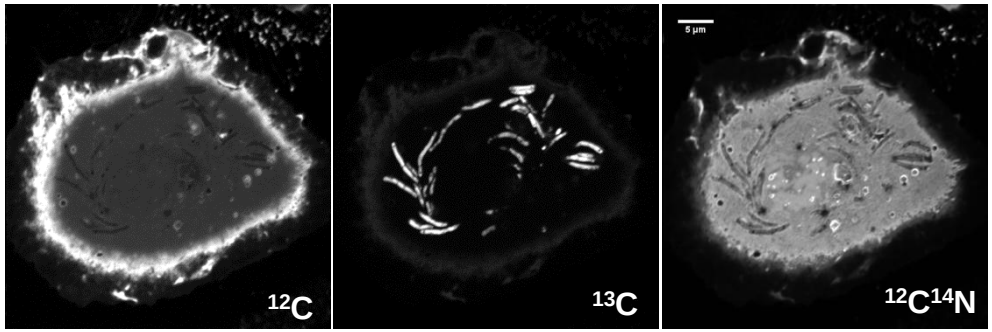
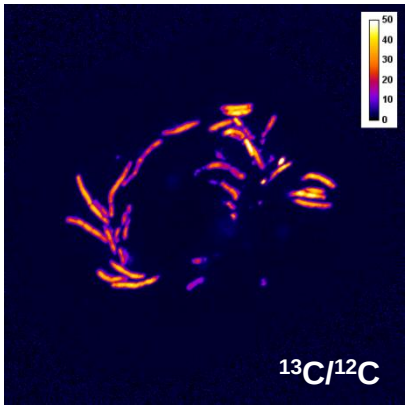
Metabolism studies :

Imaging of *Mycobacterium tuberculosis* nitrogen assimilation

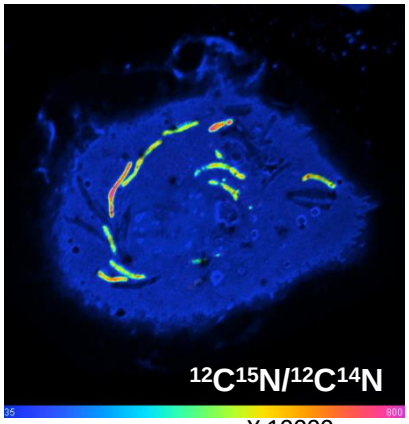


scale bar: 5 µm

Aspartate-derived nitrogen is assimilated in *M. Tuberculosis.* This process is exclusively AsnP1 dependent.



X 1000



X 10000

M. Tuberculosis could access aspartate inside its natural cellular niche and thrives inside macrophages and other phagocytes.

*Gouzy A et al, NChemBio, (2013), **9:**674-676*

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Metabolism studies :

cardiomyocyte regeneration

Claude Lechene, NRIMS/ HMS Cambridge
isotopic studies of metabolism in various tissues and pathologies

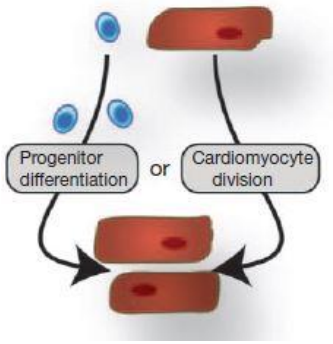
Organism

days to months

«food» enriched with labeled (^{15}N or ^{13}C) aa / nucleobase

normal diet

Question: *what is the origin of cardiomyocytes?*
Progenitor cells or pre-existing cardiomyocytes



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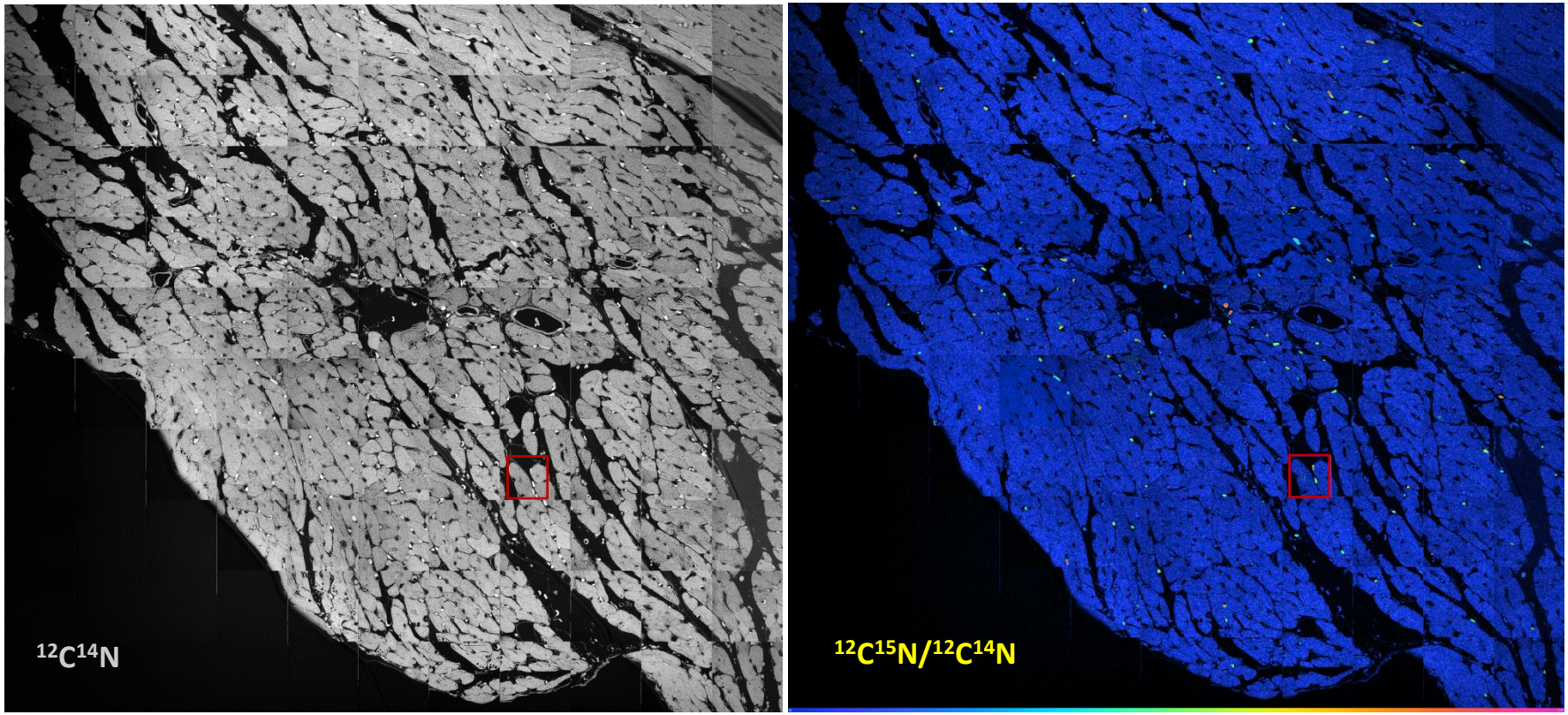
Metabolism studies :

cardiomyocyte regeneration

Mammalian heart renewal by pre-existing cardiomyocytes

*Senyo S.E et al, Nature, (2013),**493:**433-437*

Young adult



Mosaic of 121 squares (80x80 µm)

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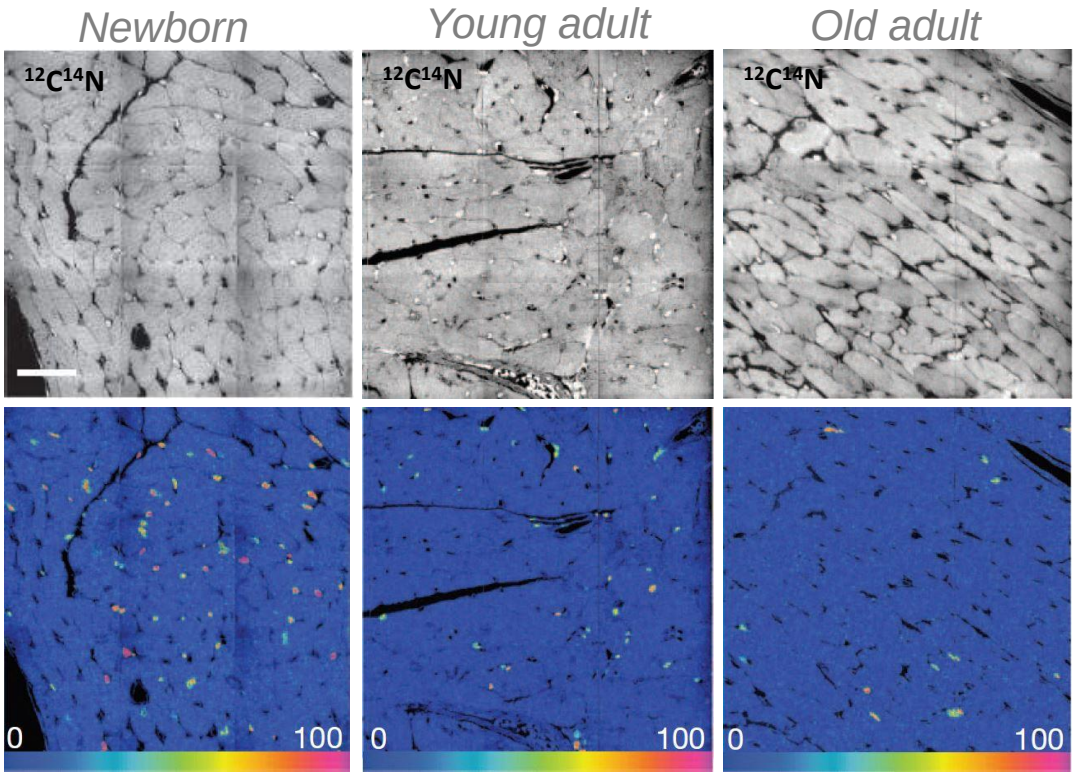
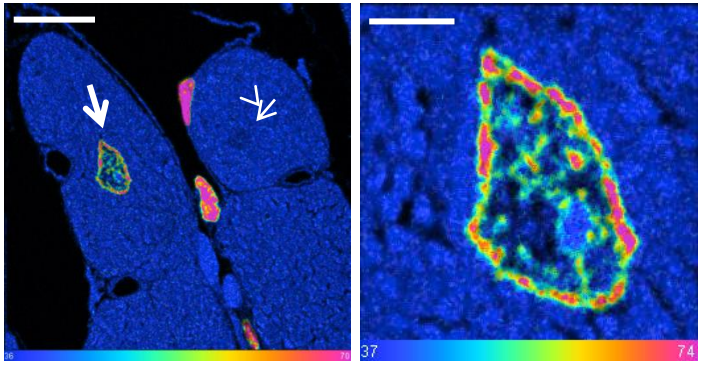
Metabolism studies :

cardiomyocyte regenearation

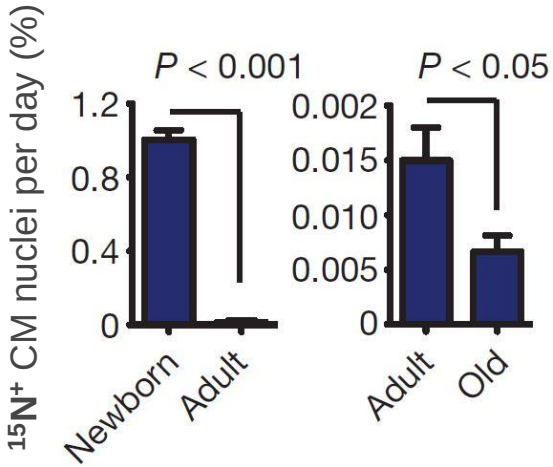


scale bar: 30 µm
scale bar: 30 µm



scale bar: 10 µm

scale bar: 2 µm



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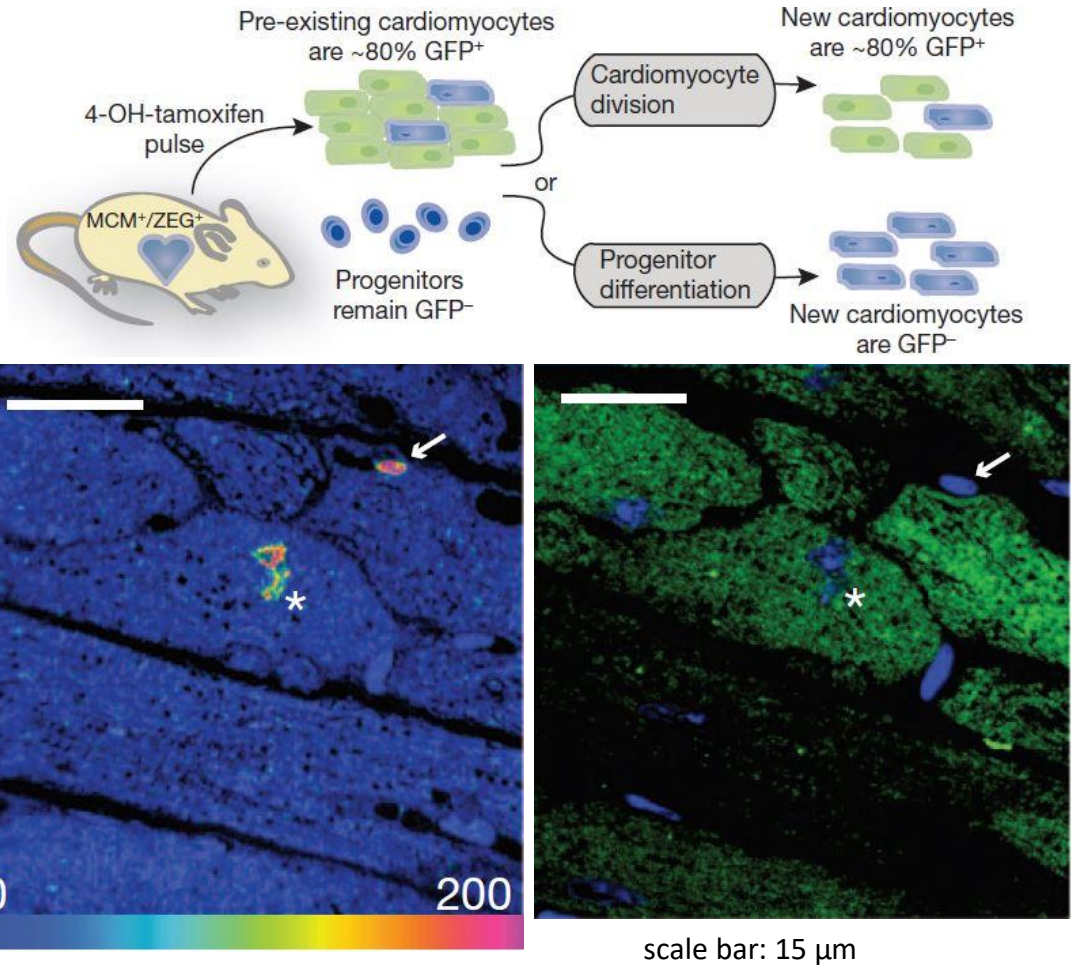
Metabolism studies :

cardiomyocyte regenaration



scale bar: 15 µm

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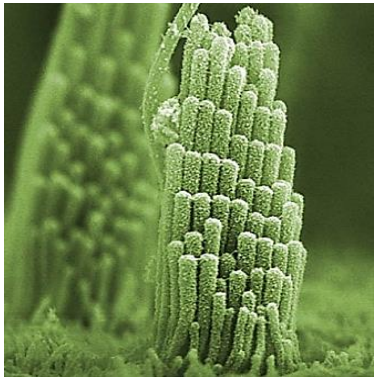
Metabolism studies :

Protein turnover in hair-cell stereocilia

Claude Lechene, NRIMS/ HMS Cambridge

Hair-cells of the inner ear are not replaced during animal's live but components must be continually renewed



Actin (about 50% of the total prot in cilia) **is replaced in 2-3 days, but how (treadmilling)?**

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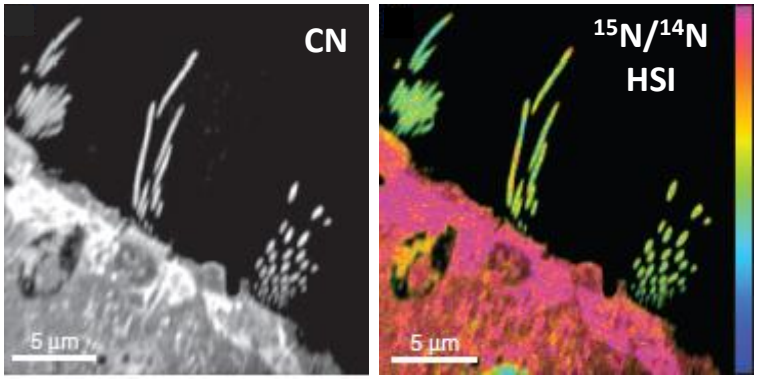
Metabolism studies :

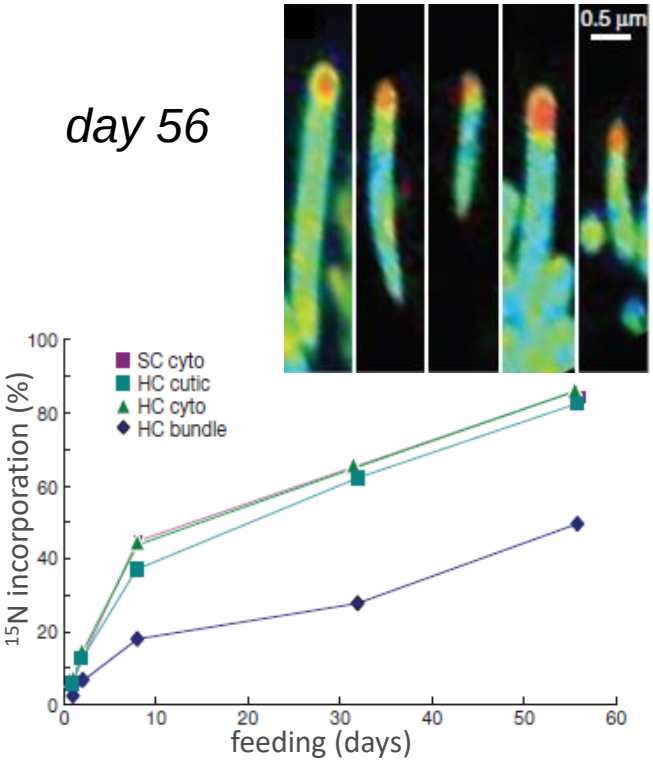
Protein turnover in hair-cell stereocilia

*Zhang DS et al, Nature, (2012),**481:**520-524*

Mice fed with ^{15}N enriched rodent chow for 1 to 150 d

^{15}N –Leu (^{15}N / ^{14}N in food ≈ 2%)





- ✓ labeling increase with days of ^{15}N feeding
- ✓ low incorporation in cilia
- ✓ protein turnover is higher in cytoplasm and tips of cilia

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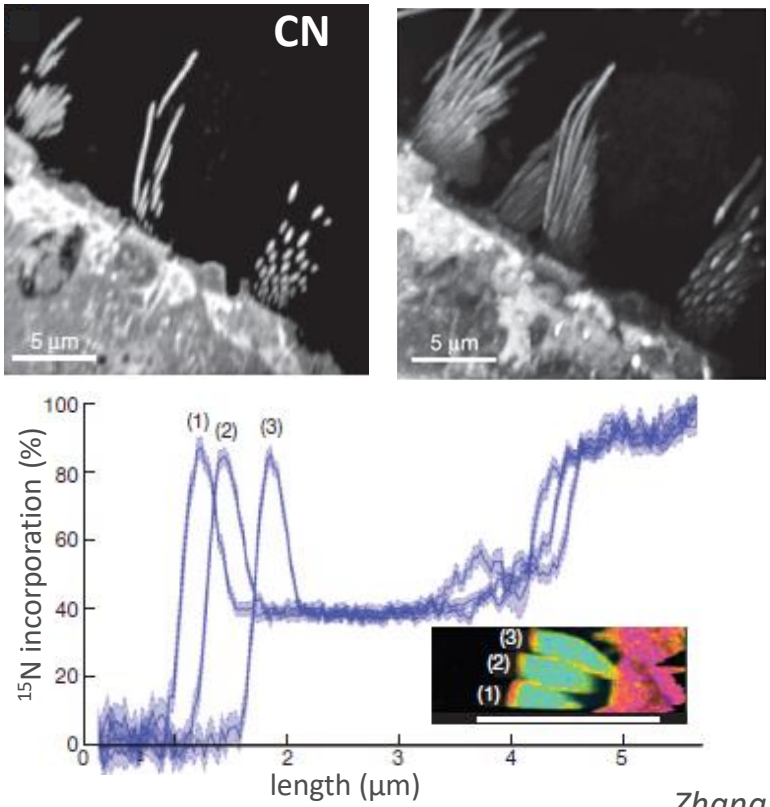
Metabolism studies :

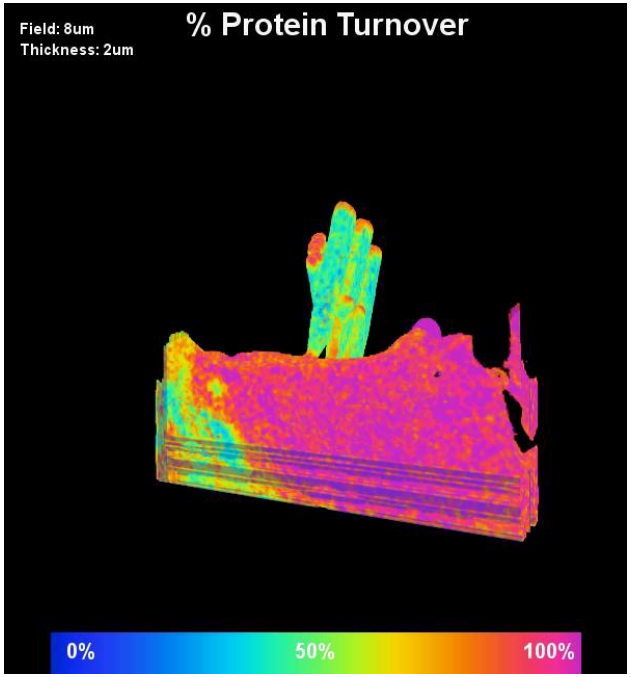
Protein turnover in hair-cell stereocilia

Mouse fed with ^{15}N-enriched food for 56 days

(^{15}N / ^{14}N in food ≈ 2%)



day 56



*Zhang DS et al, Nature, (2012),**481:**520-524*

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Application domains

Cytometry

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New insights in IHC / ICC

nature methods
Techniques for life scientists and chemists

nature medicine
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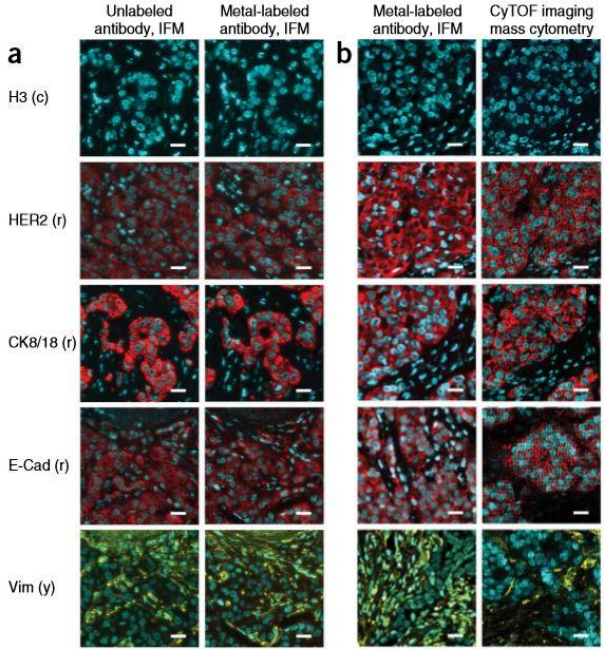
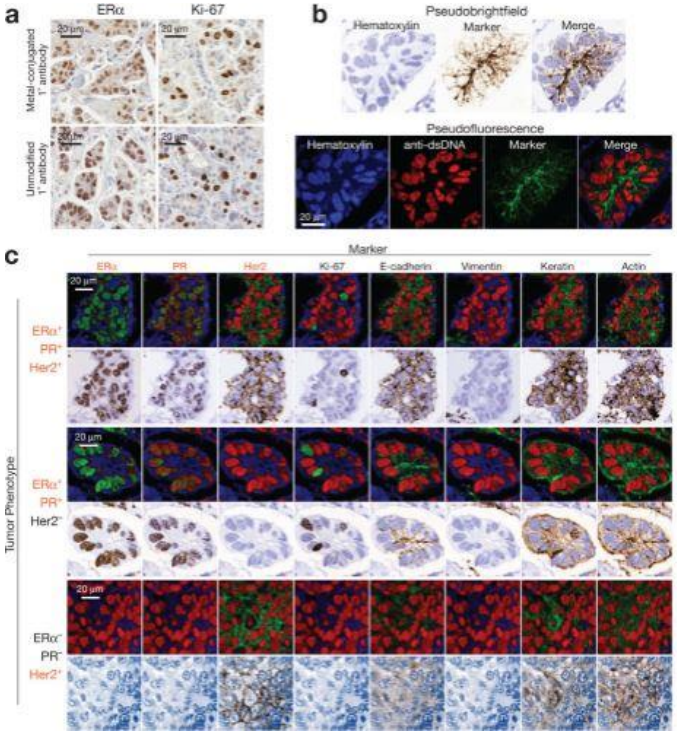
Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry

Nat Methods. *2014 Apr;11(4):417-422*

Multiplexed *ion beam imaging (MIBI) of human breast tumors*

Nat Med. *2014 April ; 20(4): 436–442*





FLUIDIGM

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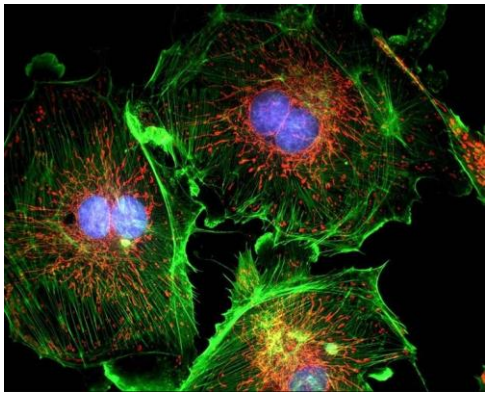
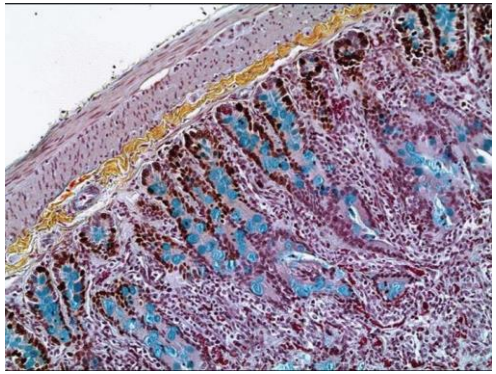
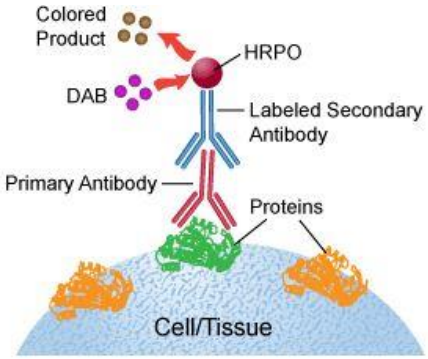
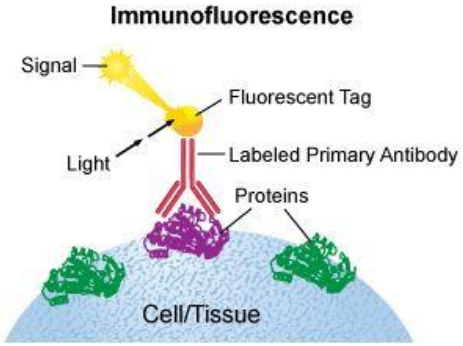
Cytometry :
fluorescence probes

ICC and IHC are used to visualize localization of specific proteins or antigens in cell or tissue









but need for multiplexed imaging increases

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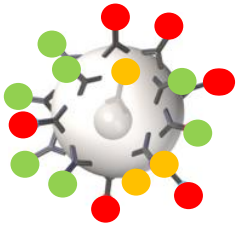
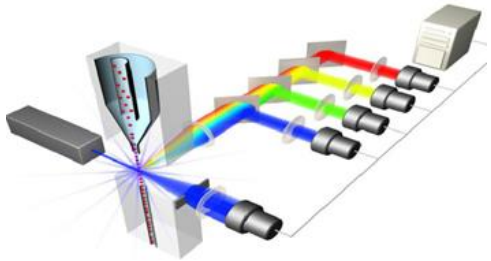
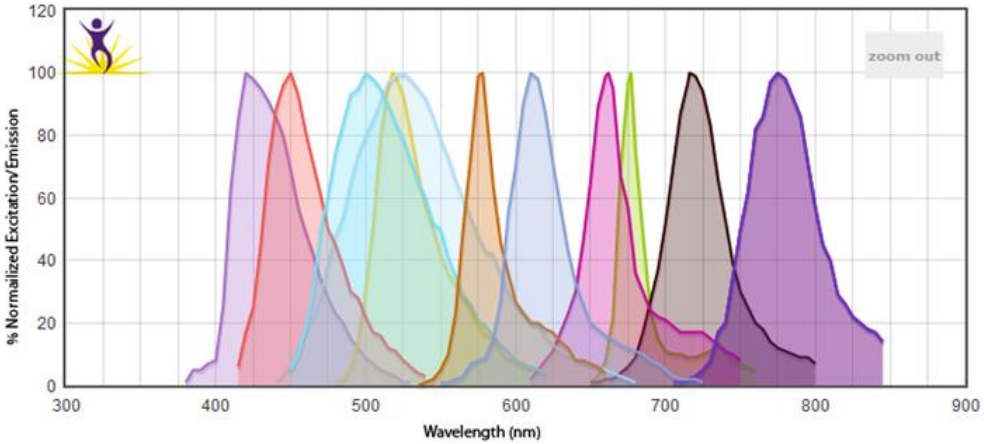
Cytometry :

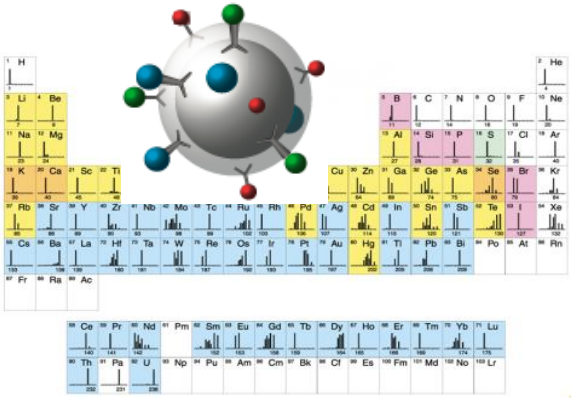
fluorescence probes vs metal ion tags

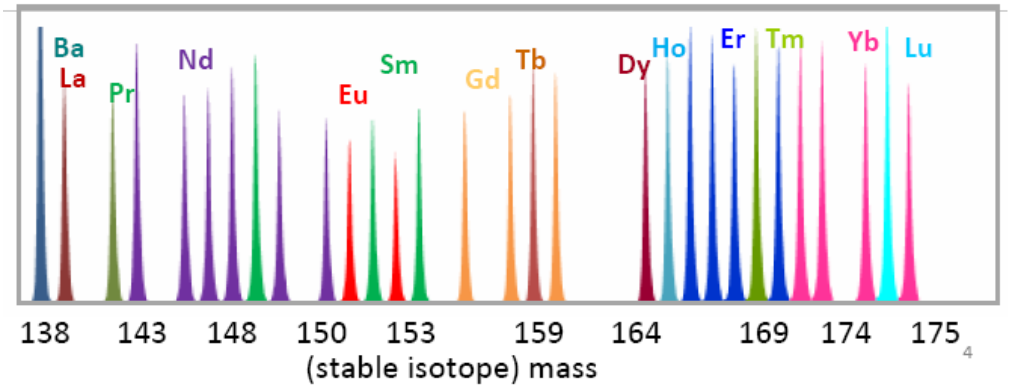






Fluorophores: signal overlap limits practical panel size

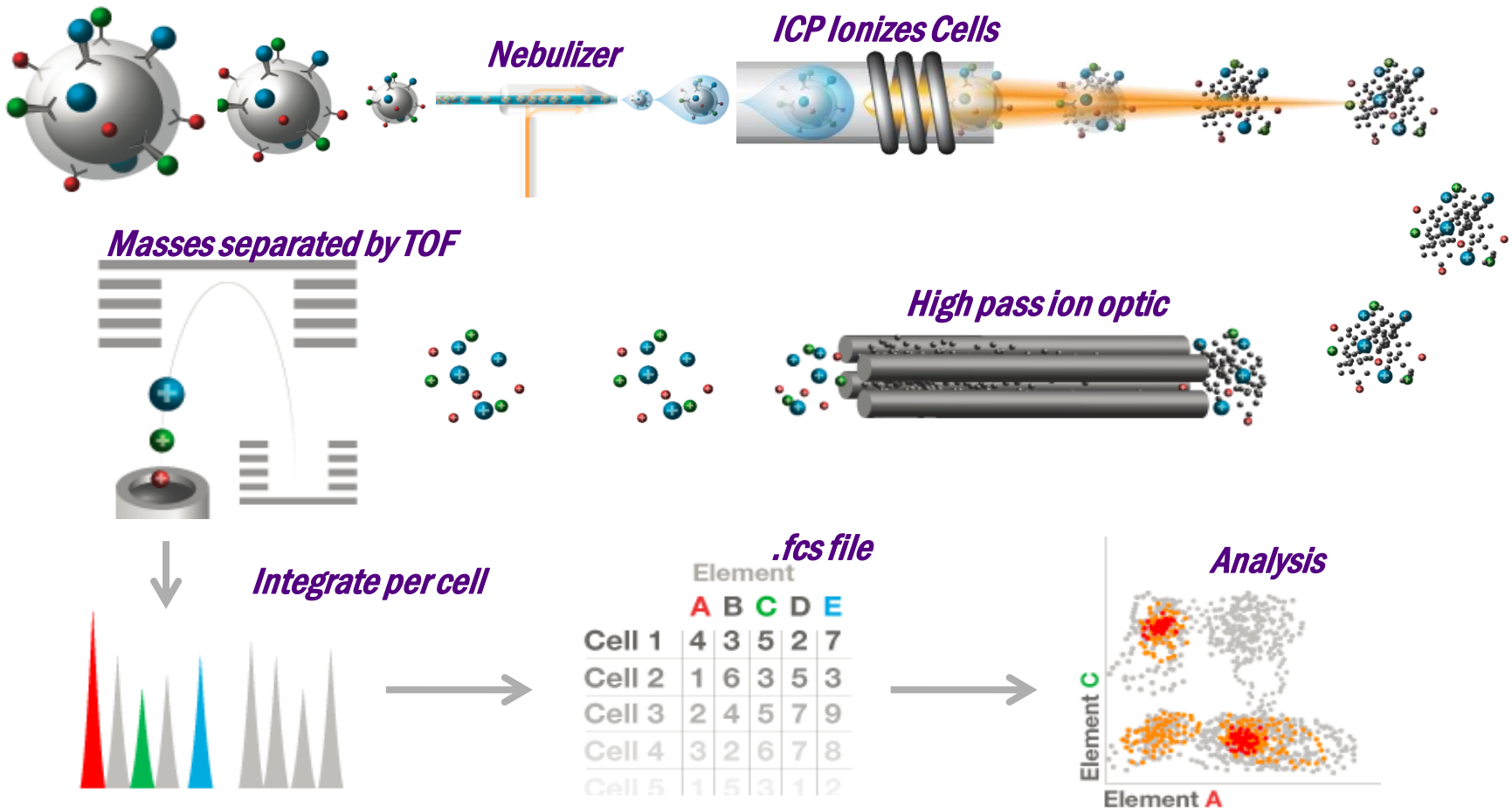




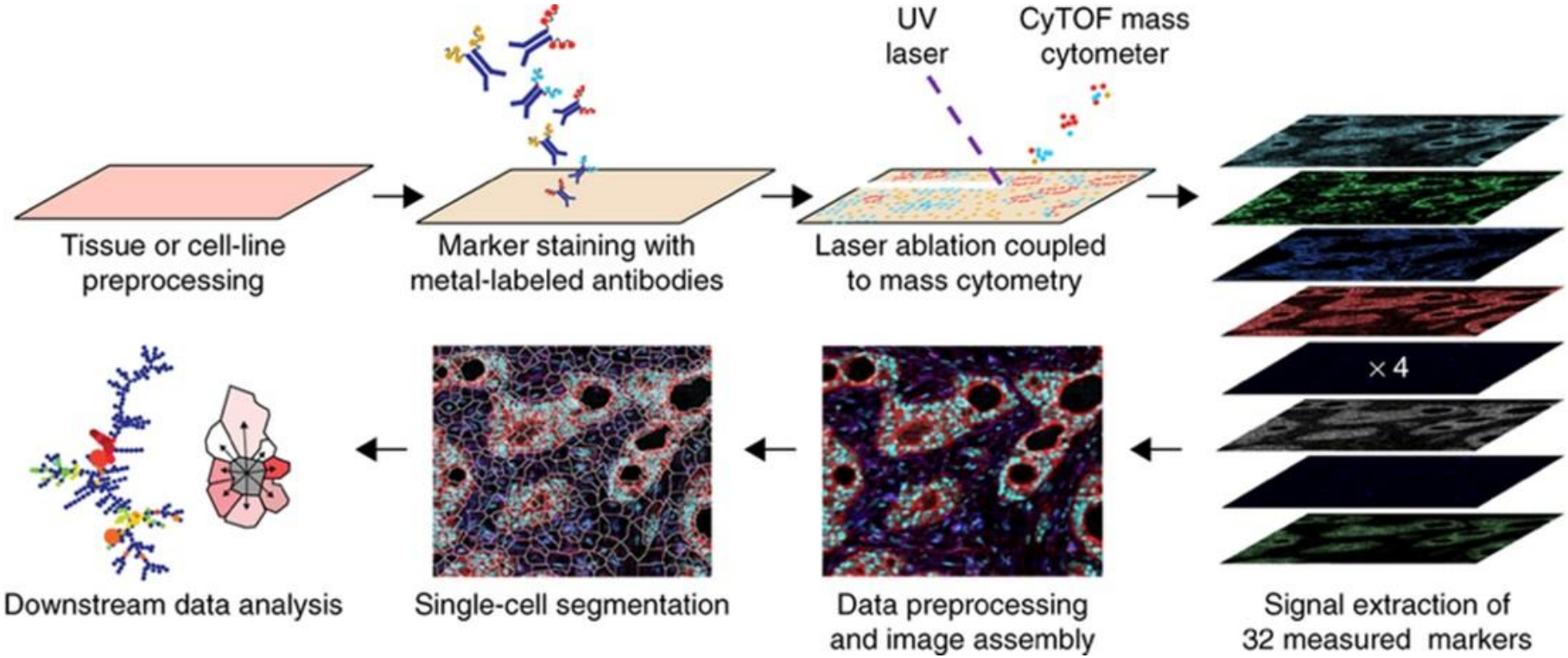
Heavy metal ion tags: mass spectrometry removes the limitation

Cytometry :

CyTOF® Mass Cytometer



Cytometry :
imaging mass cytometry



*from Giesen C. et al, Nat Methods. (2014),**11(4):**417-422*

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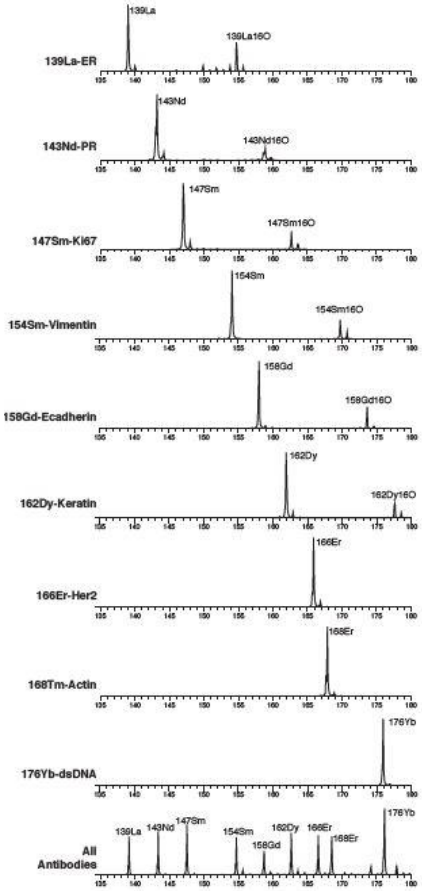
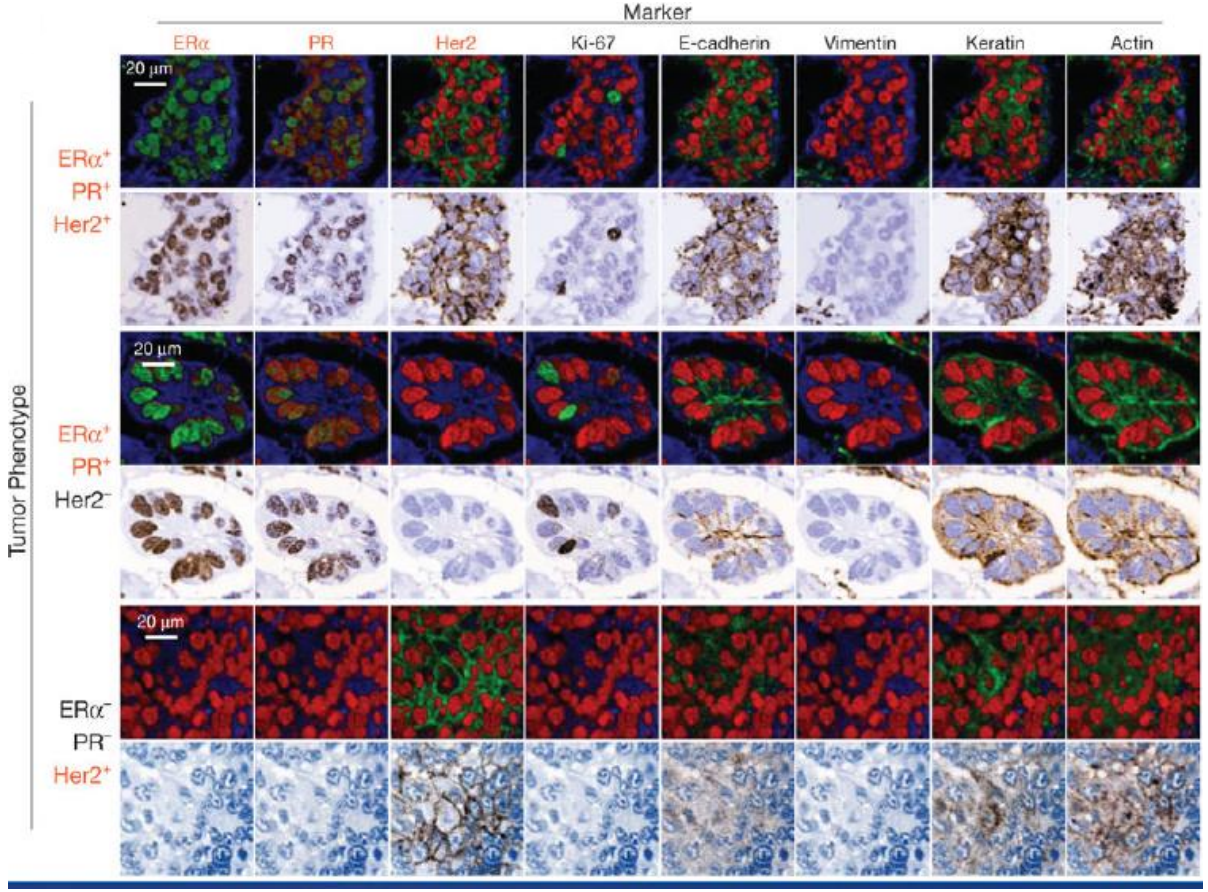
Cytometry :

multiplexed ion beam imaging





*from Angelo M. et al, Nat Med. (2014),**20(4)** : 436-442*

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