Nano secondary ion mass spectrometry (NanoSIMS) – technique and application to biological samples

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KICK-OFF MEETING
Accumulation, Subcellular Mapping and Effects of Trace Metals in Aquatic Organisms (AQUAMAPMET) **Zagreb, 14 September 2015**

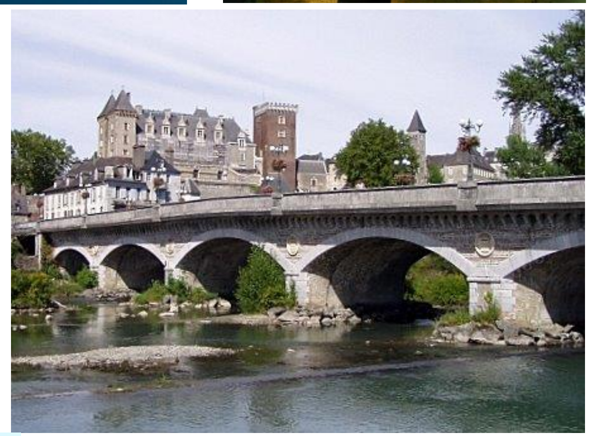





Paris
Pau



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Center of **Ma**ss Spectrometry for **R**eactivity and **S**peciation **S**ciences

MARSS

FT-MS	*NanoSIMS*
HR-MC-ICPMS	*TOF SIMS*

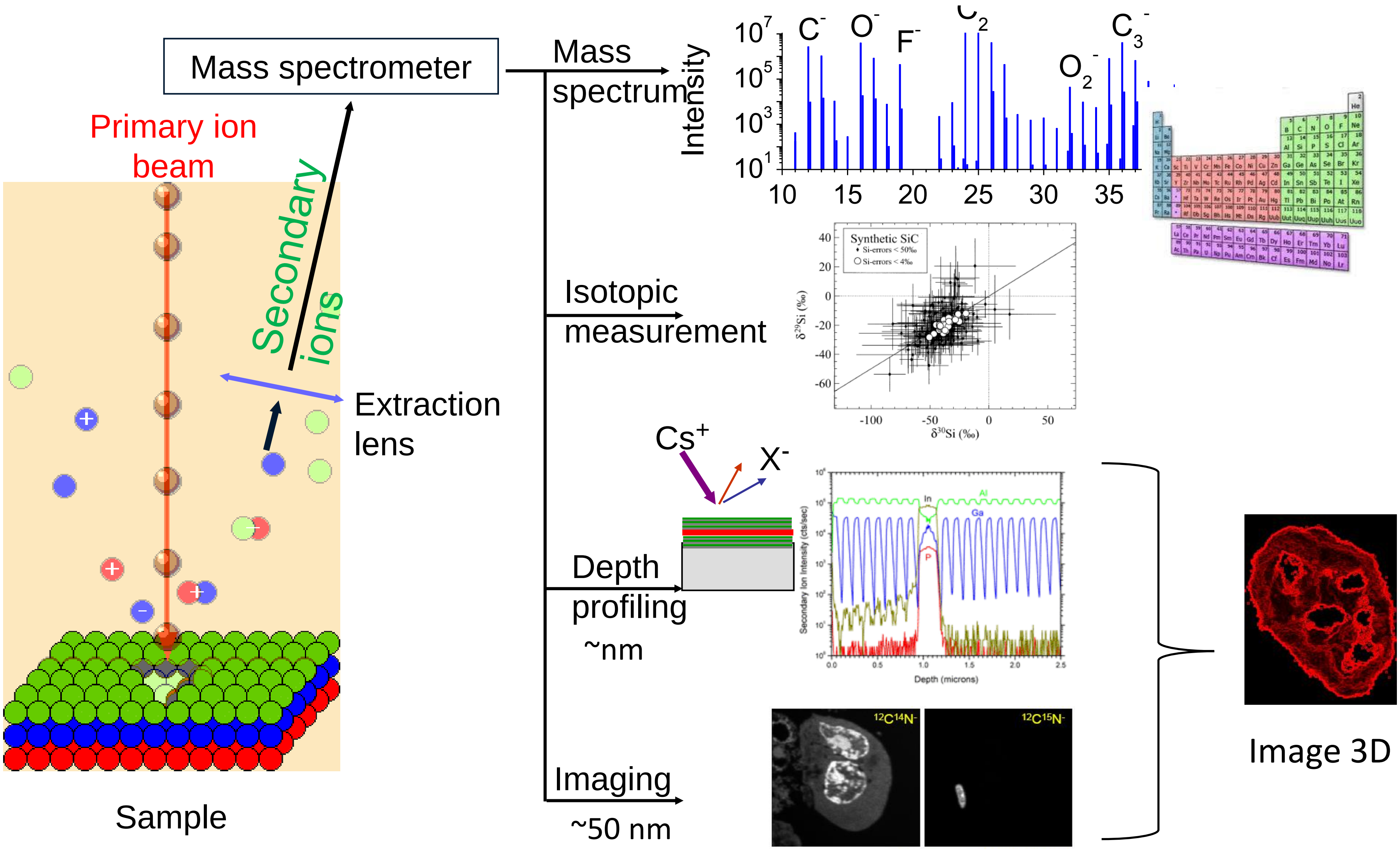
Outline

1 Introduction to SIMS

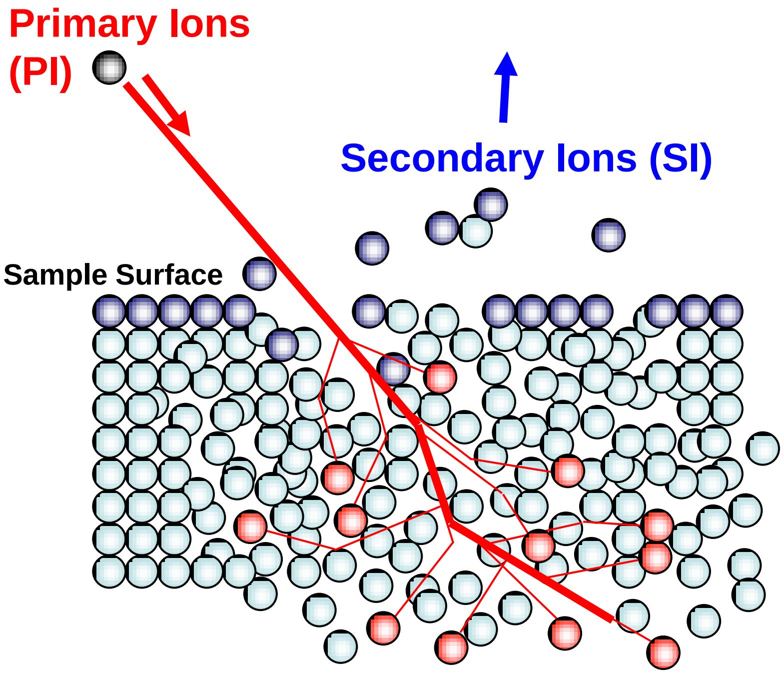
2 Element imaging by Nano Secondary Ion Mass Spectrometry (NanoSIMS)

3 Application to biological samples: subcellular element imaging

SIMS :
Secondary ion mass spectrometry



Dynamic SIMS technique



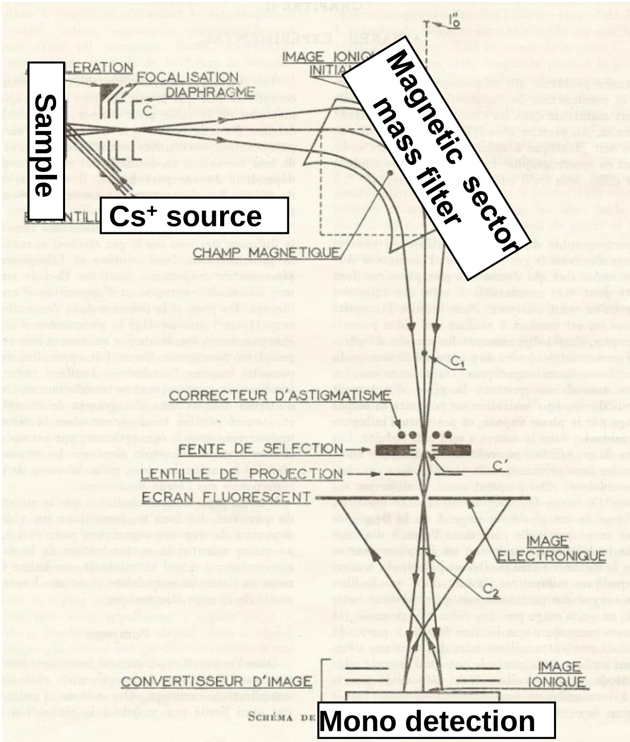
- Samples analyzed under **ultra high vacuum** (UHV); biological samples must be dehydrated.

- **Bombardment** by focused **Primary Ions** (PI):
  - **Collision cascade** (10-20nm depth) with simultaneous Implantation and Sputtering.
  - Use of **reactive PI species** to enhance the **ionization** yield (**O**$^-$ for + ions, **Cs**$^+$ for - ions)

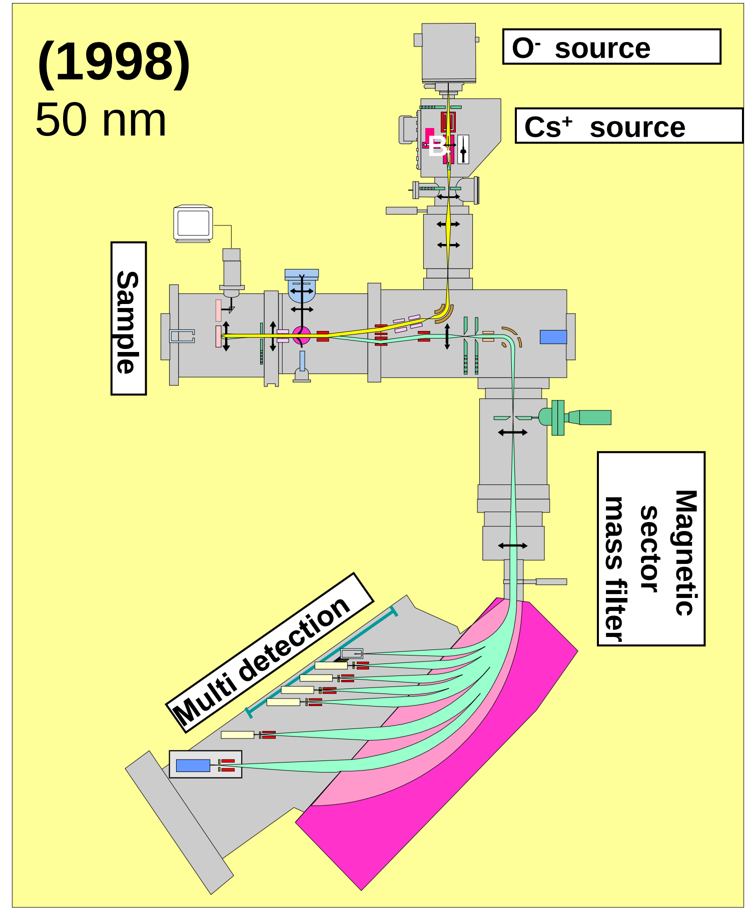
- **All molecules are broken**, single atoms and clusters are ejected.

- A **small fraction is ionized** (+ or - charge), **SI** available for **Mass Spectrometry**.

The Secondary Ions, characteristic of the local composition, are collected, then separated in a magnetic sector analyzer according to their **mass/charge** ratio: SIMS reveals **elemental** (H included) and **isotopic** surface composition



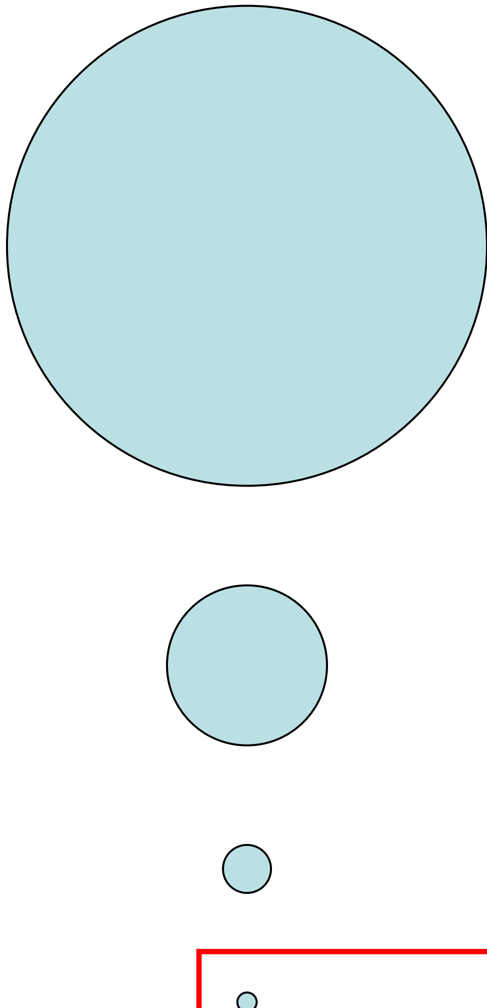
Prof. Slodzian



Cameca SMI 300, 1968

Cameca NanoSIMS50, 2000

The beam size of the microprobe



IMS 3f Ion Microprobe
2 – 3 µm — 70 ‘

IMS 4f 500 nm — 80 ‘

IMS 6f / Tof-SIMS 250 nm — 90 ‘

NanoSIMS50 50 nm — 2000 ‘

Nano Secondary Ion Mass Spectrometry (NanoSIMS)

The NanoSIMS 50L instrument
part of the new Mass Spectrometry Center in Pau **(MARSS)**

- **Reactive primary ions; lateral resolution: 50nm in Cs^+, 200nm (50nm) in O^-)**
- **Parallel Detection: 7 masses**
- **High Sensitivity *<u>together with</u>* High Mass Resolution *<u>and</u>* small spot size**

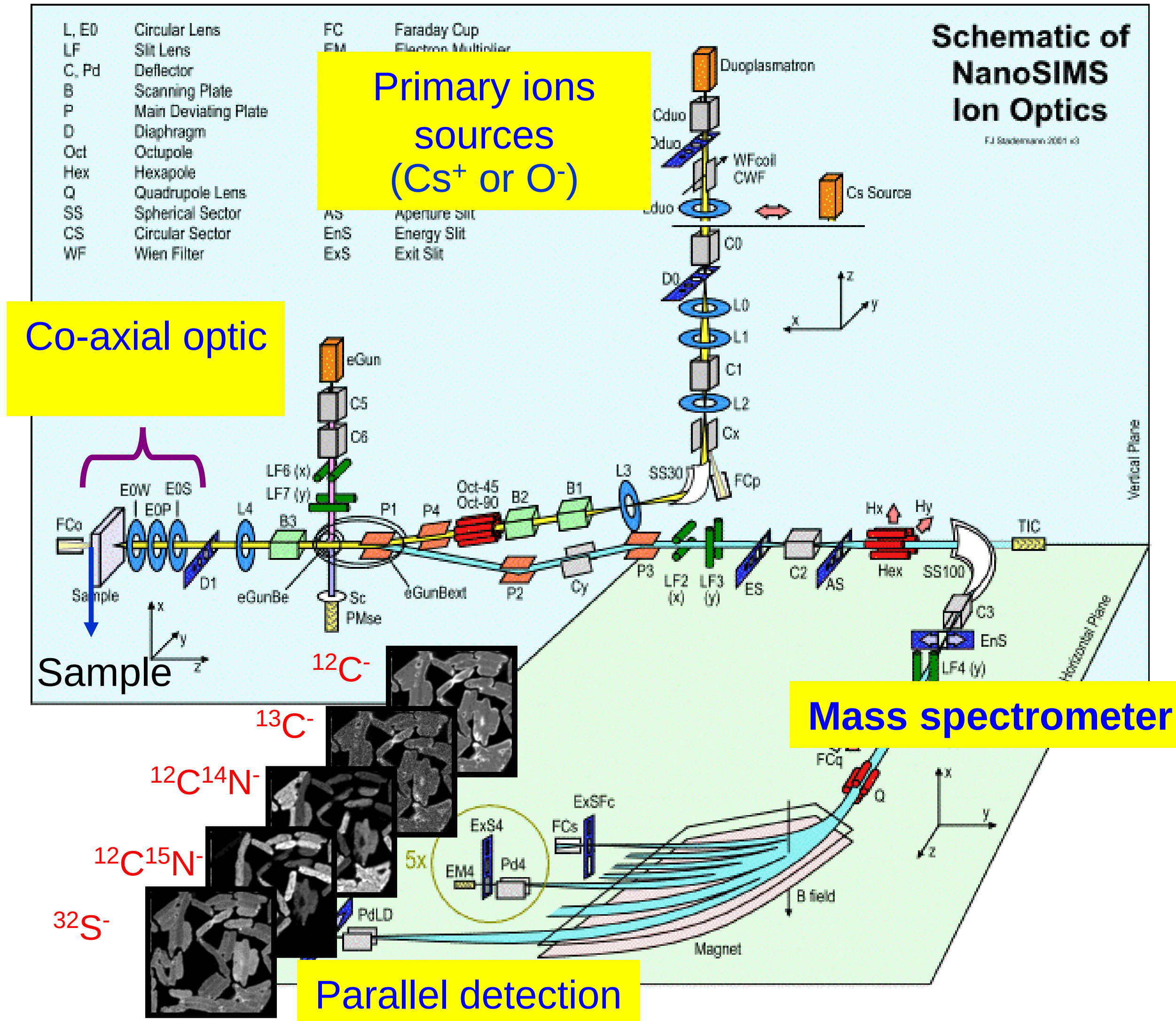
Original design by Pr. Slodzian/ University Paris Sud, Orsay. Developed in collaboration by UPS Orsay, ONERA and CAMECA.

NanoSIMS : Ionic microprobe

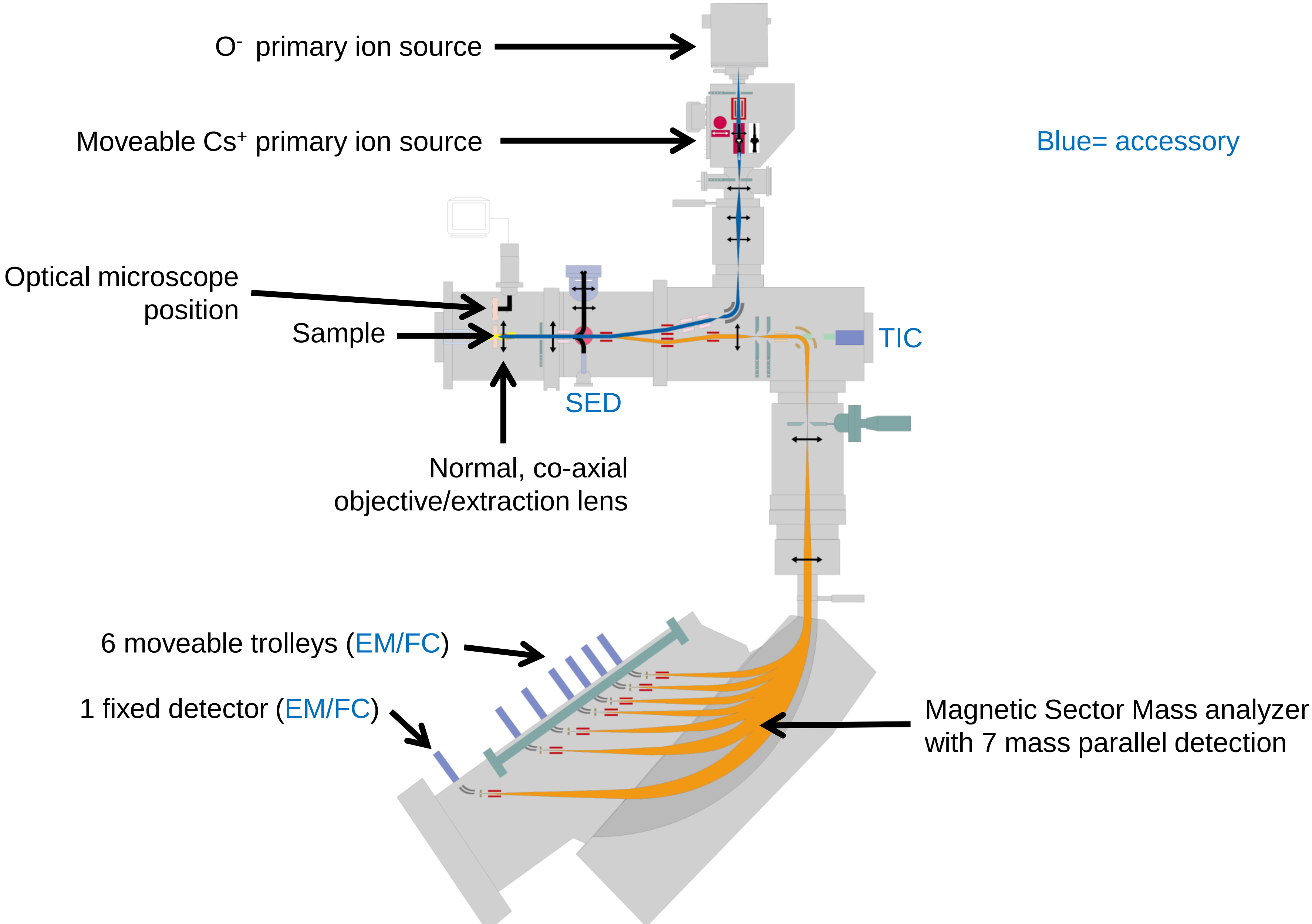
(http://presolar.wustl.edu/nanosims/schematic.html)

Analysis by scanning of a fine probe (50nm with Cs^+)

Parallel ions counting and reconstruction of the elemental distribution (and isotopic)



NanoSIMS 50L scheme



SIMS Signal : Secondary ion intensity

$$I_{ionM} = T \, . \, Y_i \, . \, d_b \, . \, S \, . \, Sy \, . \, [M]$$

d_b: bulk density,

Surface,

Sy: Rdt Ion I/II

T: instrumental transmission

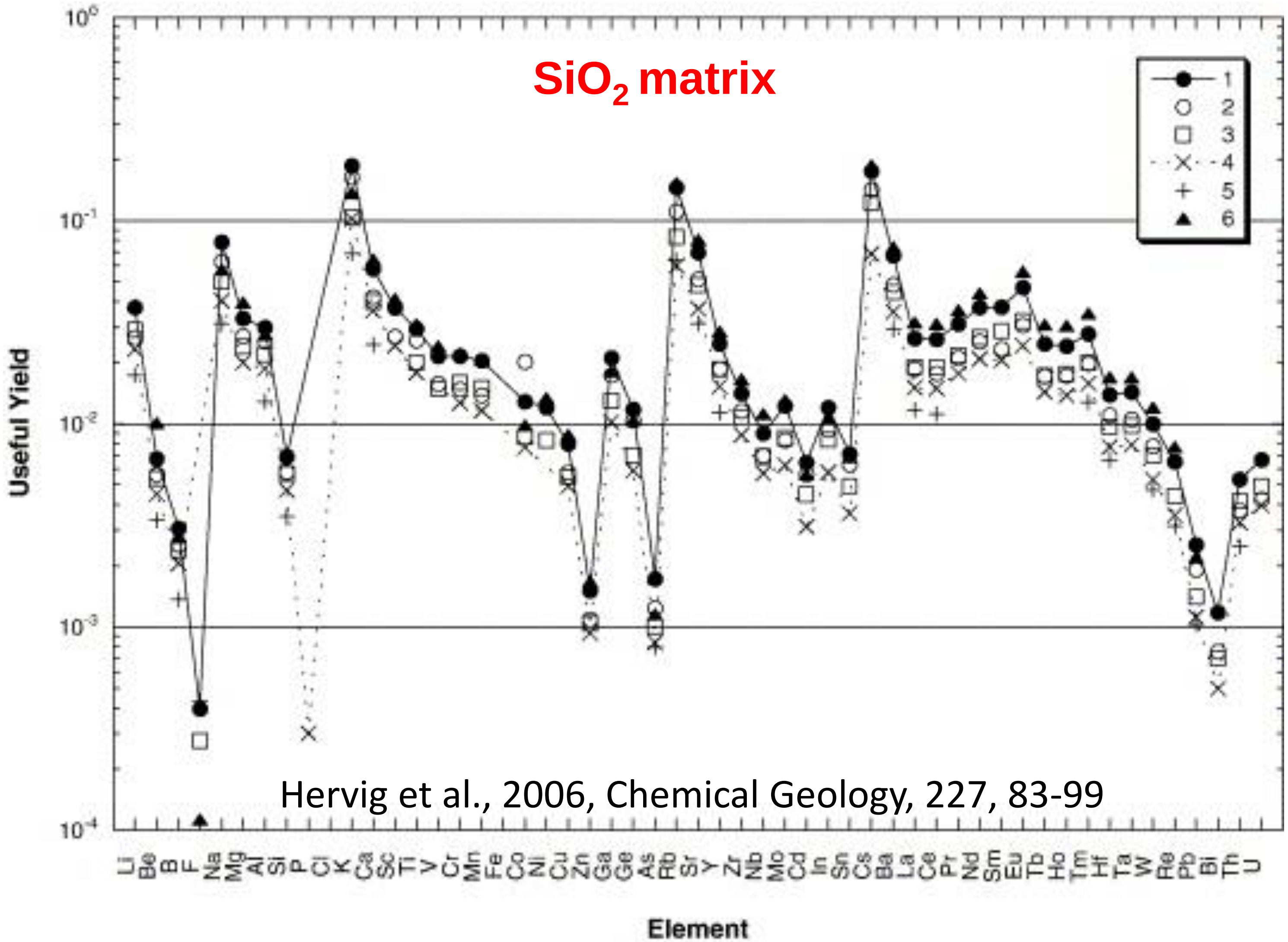
Y_i: ion yield of M_i $\dfrac{\text{nb ions } \textbf{ionized}}{\text{nb atoms sputterized}}$

The SIMS ionization efficiency is called ion yield, defined as the fraction of sputtered atoms that become ionized.

T. Y_i = UY Useful Yield

$\dfrac{\text{nb ions } \textbf{detected}}{\text{nb atoms sputterized}}$

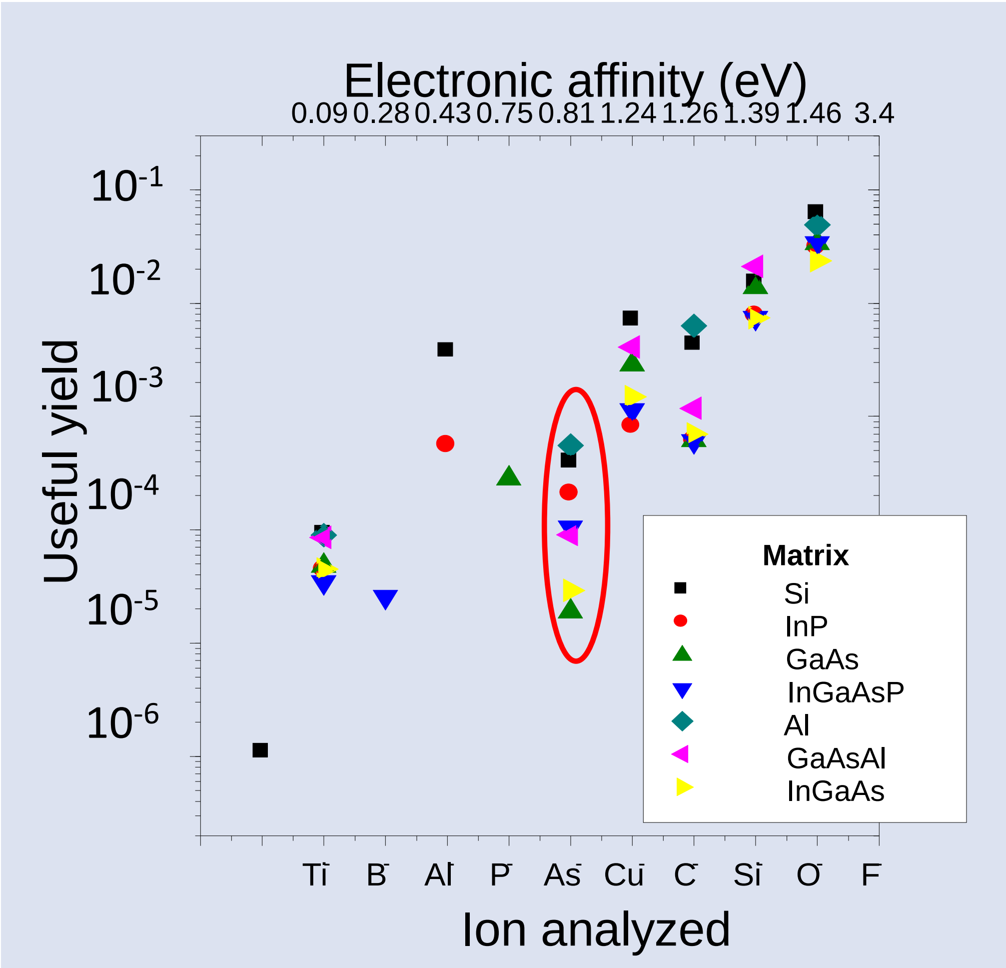
Useful Yield vs element



UY ^{12}C $= UY\ ^{13}C$

Isotopic ratio not affected by the UY

Useful Yield vs matrix



UY Useful Yield

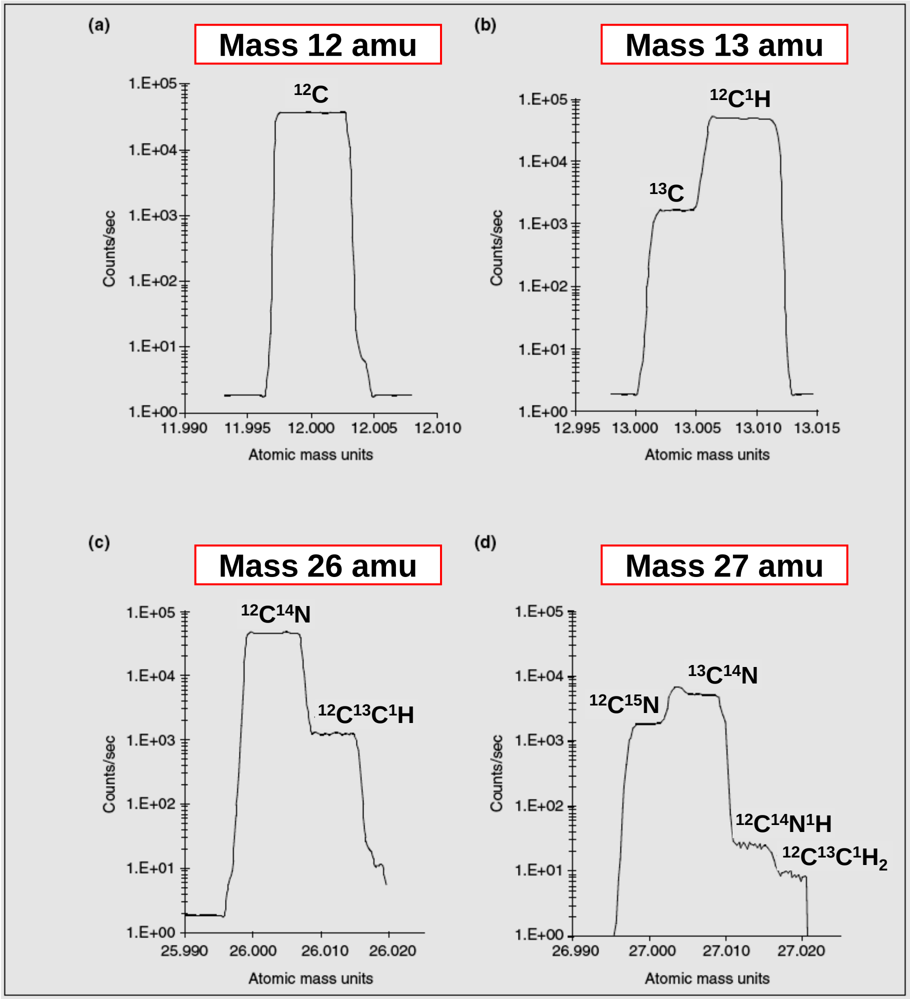
$$\frac{\text{nb ions detected}}{\text{nb atoms sputterized}}$$

Isotopic ratio not affected by the UY

Matrix effect
→ Quantification : difficult

Philipp,P. et al., Int. J, of Mass Spec. 2006

High Mass Resolution need in SIMS



Section of embedded biological tissue.

In SIMS mass interferences are usually present at each unit mass.

High Mass Resolution is necessary to resolve such mass interference, specially for precise isotope ratios, quantitative measurements and trace level detection.

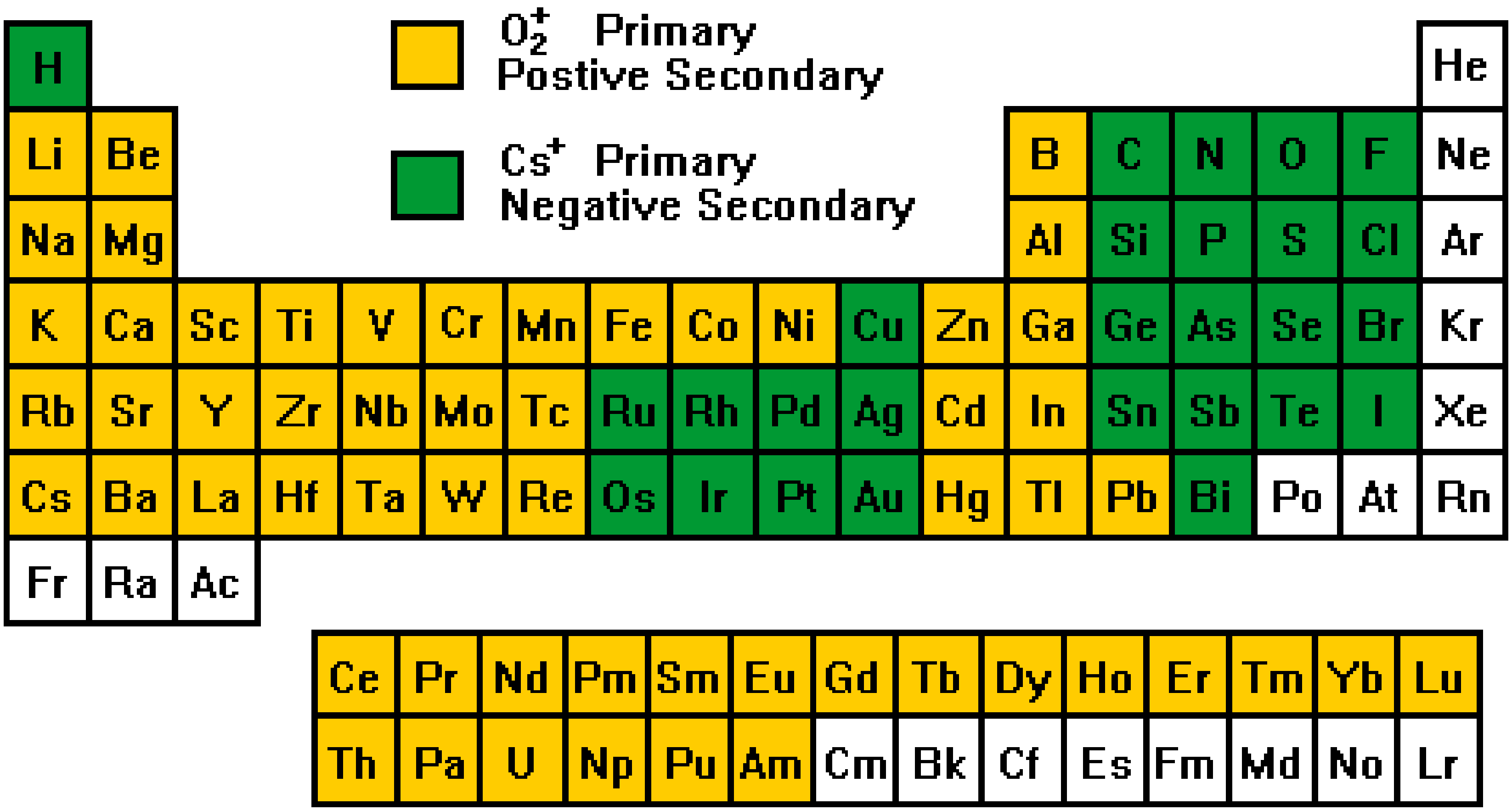
Flat top peak mode is used for better isotope ratio precision and reproducibility.

Note the dynamic range (log. scale) and peak shape.

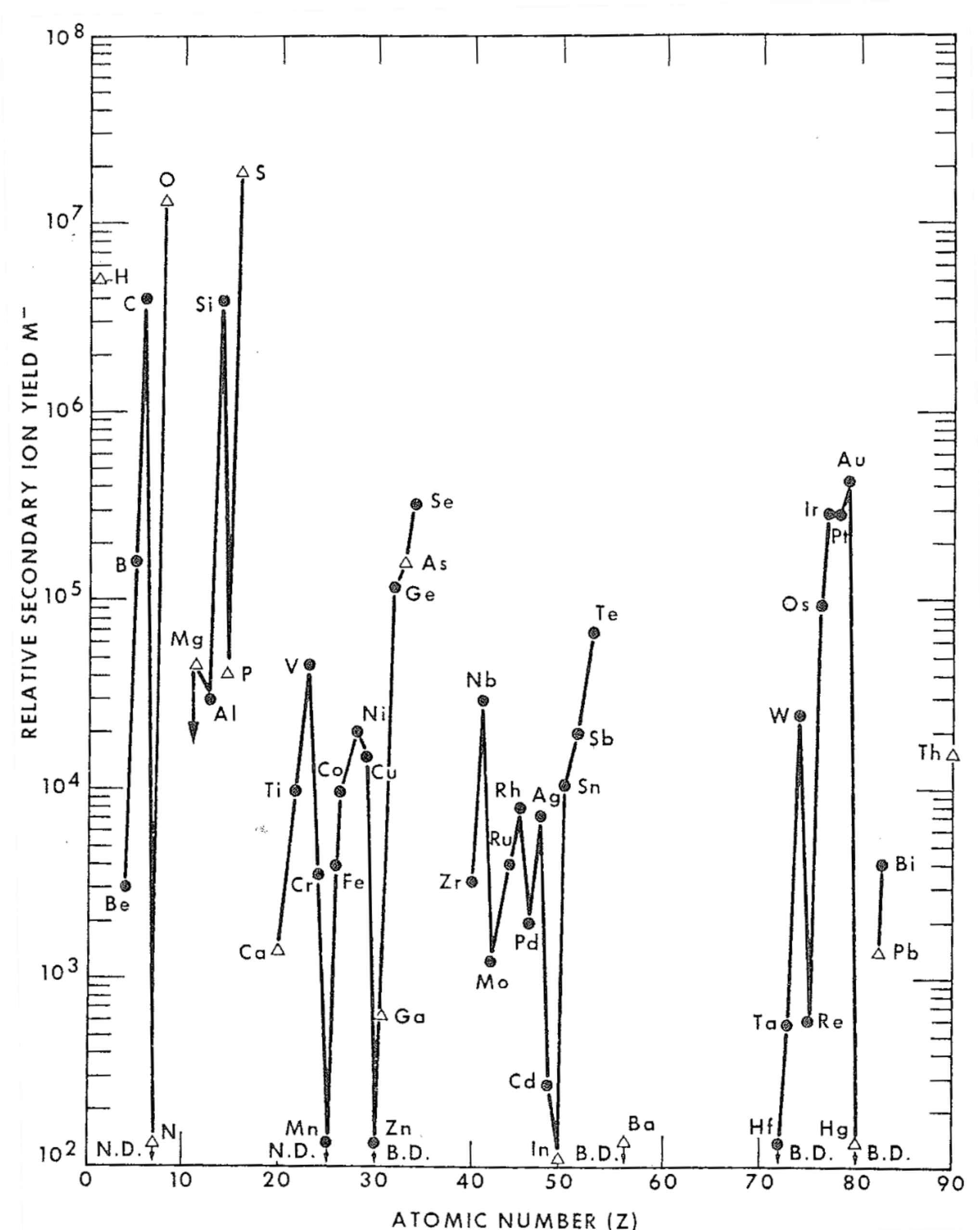
The uniqueness of the NanoSIMS is to keep nearly full Transmission (= High Sensitivity; <u>ppm level</u> for most elements) at High Mass Resolution (<u>M/ΔM = 5000</u>) *together* with High Lateral Resolution (<u>< 50nm</u>).

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

Secondary Ion Yields -- Primary Beam Effects



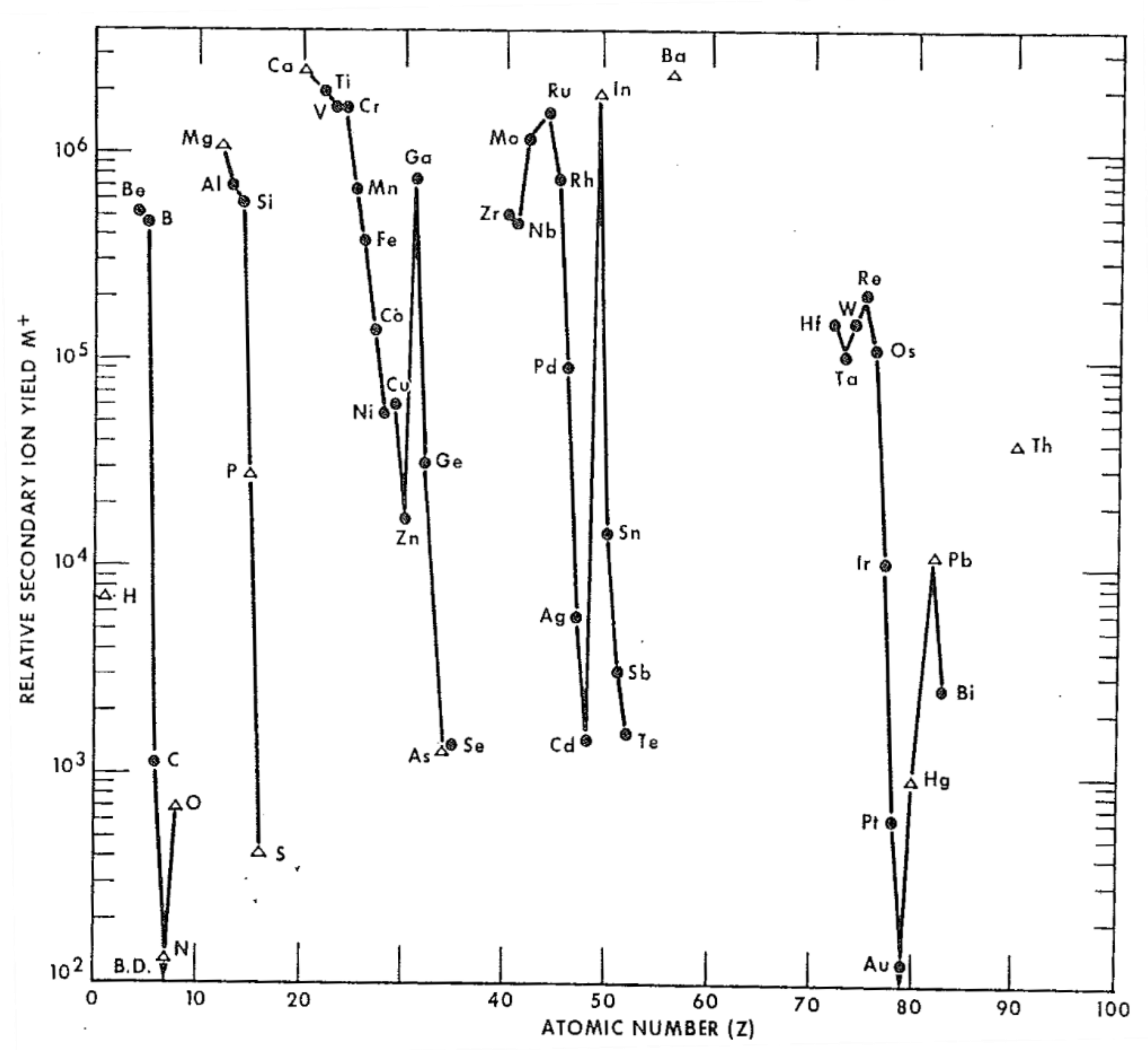
Relative negative ion yield for a Cs$^+$ ion source in SIMS



Classic NanoSIMS application for cell imaging:
C, N (via CN$^-$), **O, S, P, Se** and their stable isotopes for tracer studies.

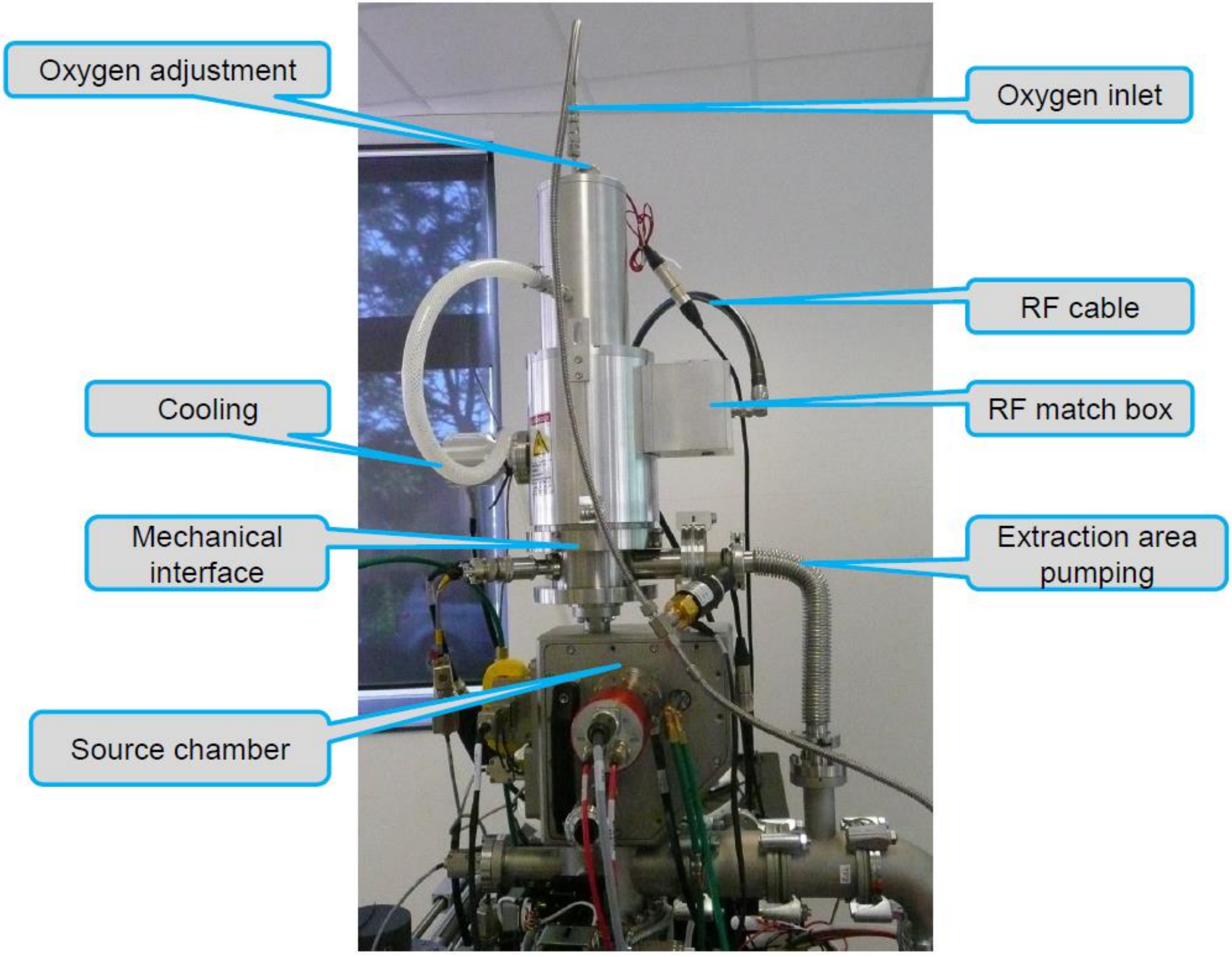
Storms, H.A., K.F. Brown, and J.D. Stein *Evaluation of a Cesium Positive-Ion Source for Secondary Ion Mass-Spectrometry*. Analytical Chemistry, 1977. **49**(13): p. 2023-2030.

Relative positive ion yield for a O- ion source in SIMS

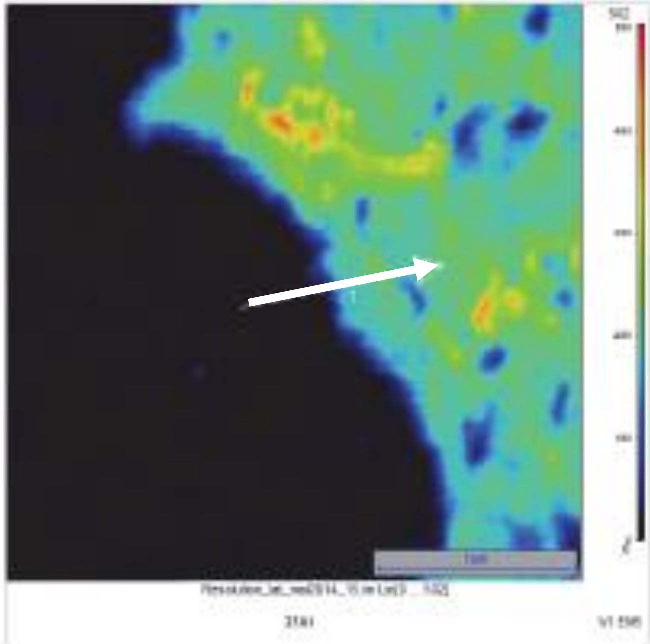


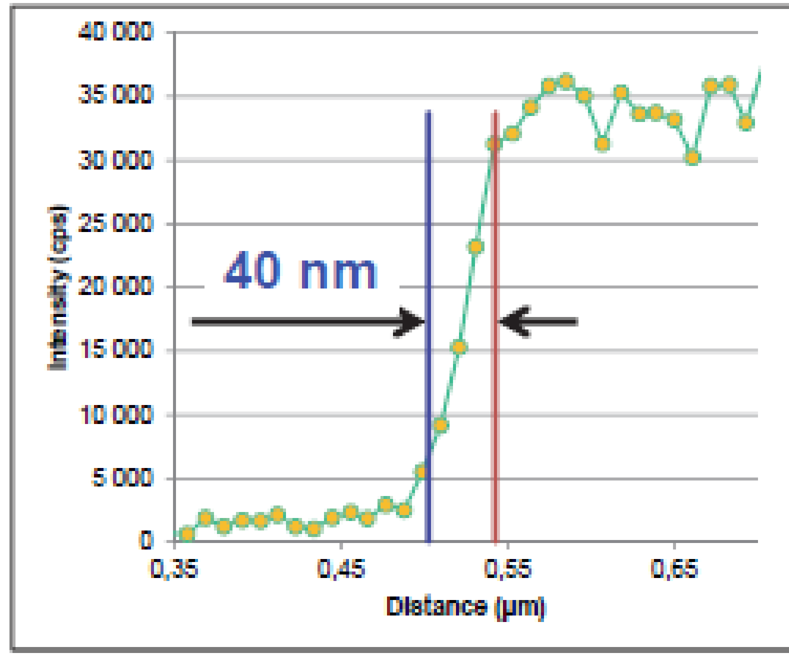
Imaging of major
And trace elements
should be possible:
Ca, Mg, Mn, Cr, Cu
…

New O- source developed on our instrument by CAMECA



Determination of the size of the O^- primary ion beam (probe size)



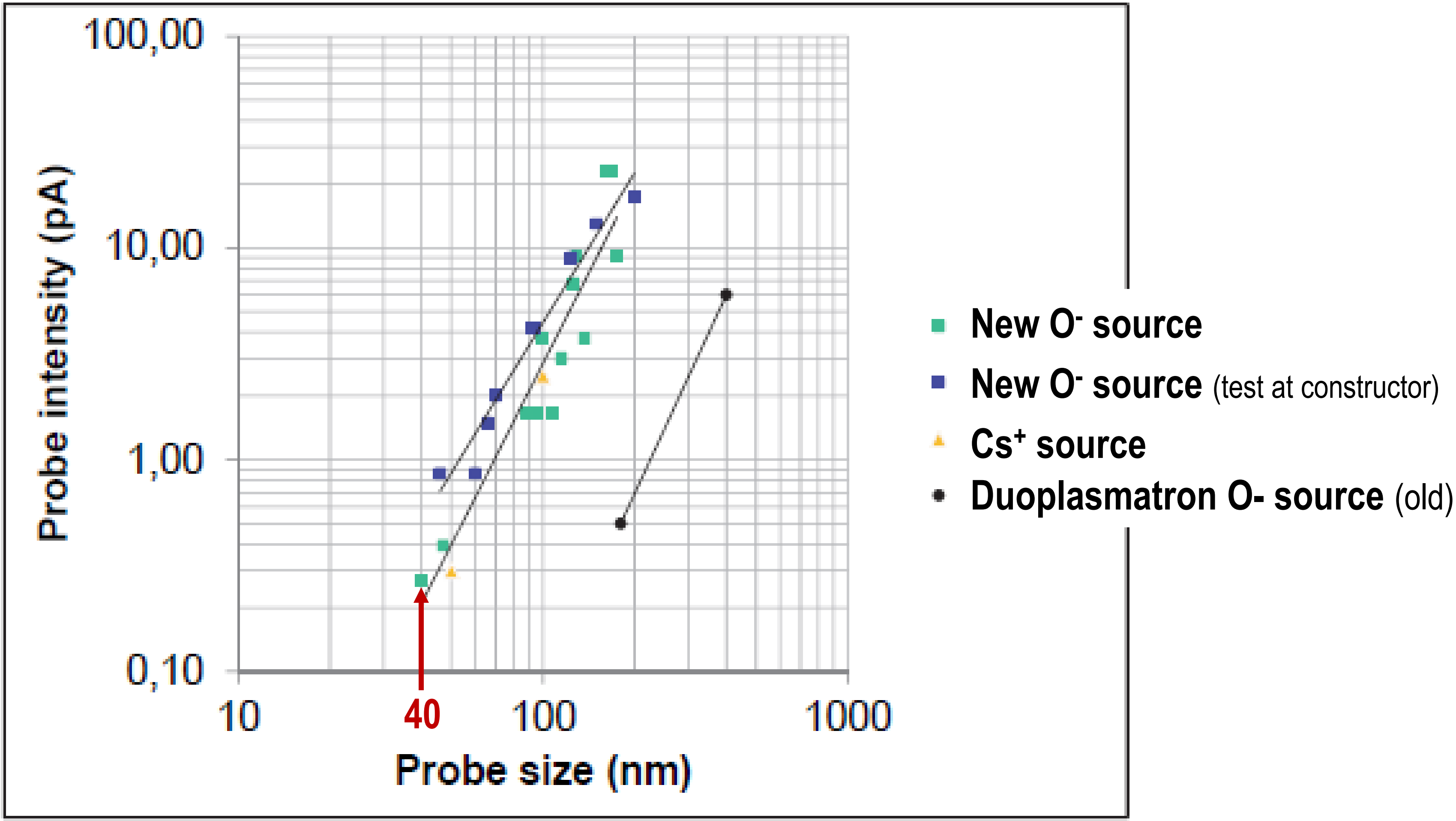


Al/Si oxide grain sample

Image size: **3 x 3 µm**
Probe size: **40 nm** ***(16-84%)***
Probe intensity: **0.3 pA**

Line scan (left image) showing **intensity variation** from **16 to 84 %:** determination of **probe size** (resolution)

Comparison of NanoSIMS primary ion sources

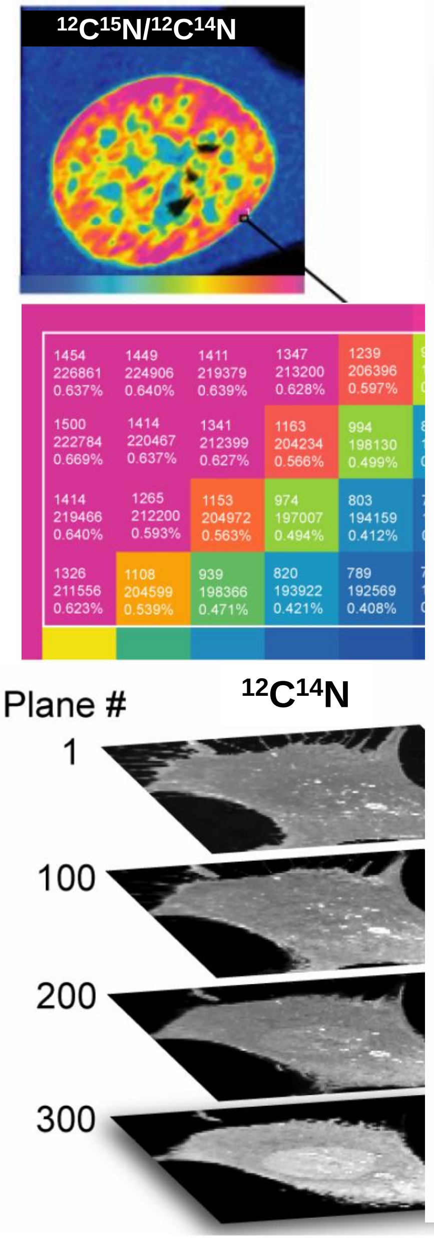


The new oxygen ion source show similar sensitivity and even better resolution **(40 nm)** as the cesium ion source and by far better characteristics (resolution and sensitivity) than the old oxygen ion source.

Advantages of the new O^- source

- **Higher beam density** = better sensitivity for metals (Ca, Fe, Cu, Mn….)
- **Higher lateral resolution** than conventional Oxygen sources = sharper images enabling the observation of smaller details
- **Less maintenance** = less instrument downtime
- **Stability:** < 1.6 % over 14h; **lifetime:** > 1000h (up to now)

The NanoSIMS: a scanning Ion Microprobe with a multicollection mass spectrometer



Ultra High Vacuum (➔ dehydrated sample)

Ratio pixel by pixel

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

Comparison of different MS imaging techniques

Organic information

Mass resolving power

	Dynamic SIMS	Static SIMS	(MA)LDI-TOFMS	FT-MS (MALDI)
Data	Elements	Elements + organic fragments	Pseudo-molecular (low fragmentation)	Pseudo molecular (low fragmentation)
Lateral resolution	50 – 100 nm	200 nm - 10 mm	10 - 50 mm	50 - 200 mm
Field of View	80 /600 mm	9 cm	15 cm	n.a.
Typical mass range	< 250 Da	< 1 000 Da	> 100 000	> 10 000
Mass resolving Power	15 000	10 000 (28 Da)	20 000 (2000 Da)	> 1 000 000 (1000 Da)

Lateral resolution

Sensitivity

Fragmentation

The ultimate microscope

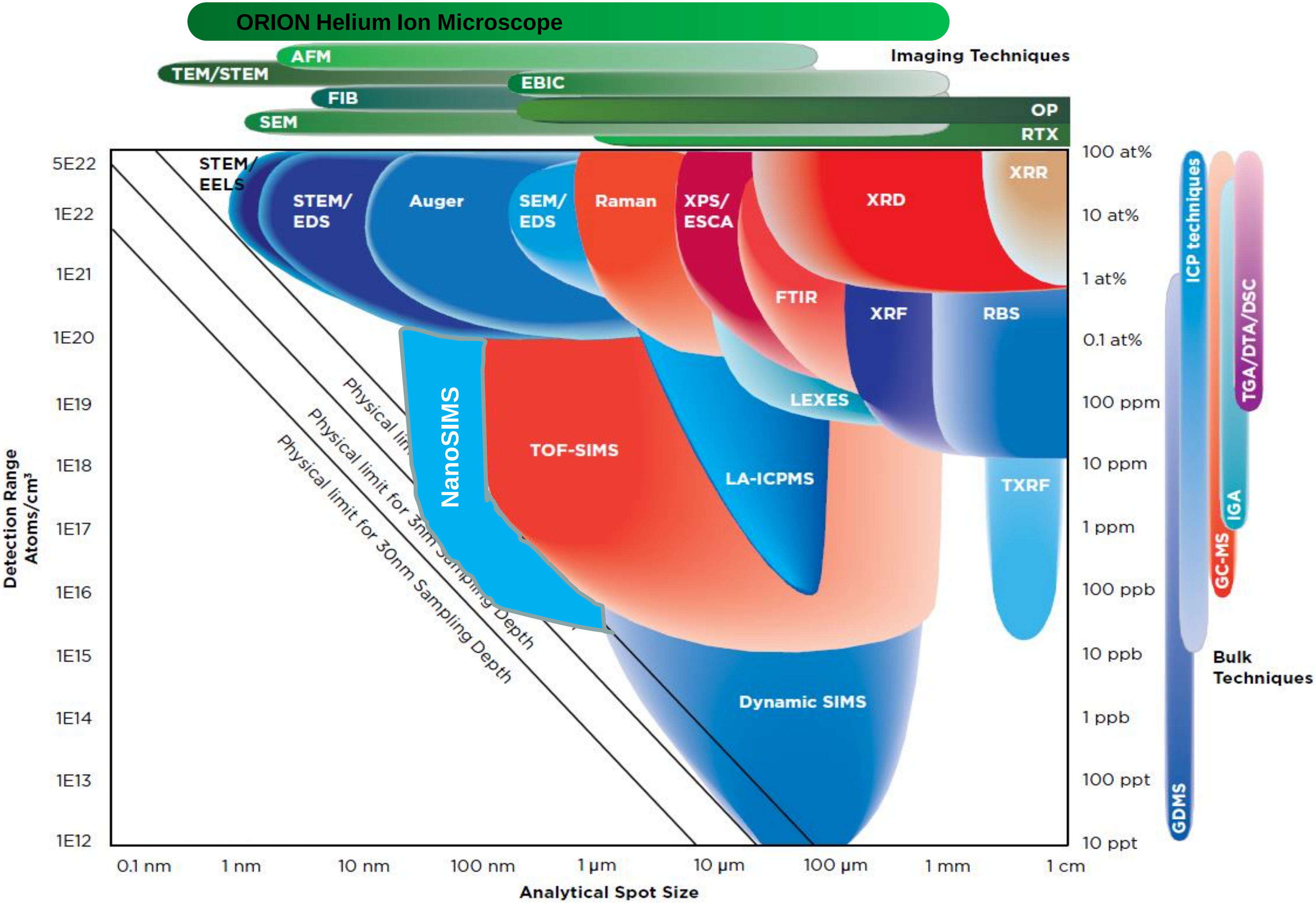


Figure: by curtesy from **Jean-Nicolas Audinot**, Centre de Recherche Gabriel Lippmann, Luxembourg

Strong points of NanoSIMS

- **Allmost all Elements** (from H, D, T,… up to Pu)
- **Very High Sensitivity**: down to ppb in spot analysis, ppm in imaging,
- **Quantification possible**, but difficult (Relative Sensitivity Factors)
- **Isotopic composition** (signature of origin and history in **astrophysics**, of past climate and origin and history in **geology**, of path and activity in **biology**, of path in **materials**)
- **Localized,** micro-analysis: down to 50nm lateral resolution (NanoSIMS), access to **3D** analysis with depth resolution of 10-15nm with NanoSIMS.
- Large **sample size** is possible for navigation and finding areas of interest
- Minimal **sample preparation** for solids (polishing and metal coating for minerals), dehydration or **TEM-like preparation for biological tissues**.

Biological applications

Use of the novel oxygen primary ion source for the localization of major (**Na, Ca, Mg**) and trace (**Fe, Cu, Mn, Zn**) metals Involved in physiological processes in plant cells

Biological sample preparation

TEM-like preparation (transmission electron microscopy)

The sample is analyzed at room temperature **under vacuum**

It must be **dehydrated and fixated**.

Flat samples are required. *Thin sections* (300-400nm) are preferable to avoid sample charging under ion bombardment. **Ultramicrotome**.

Sample preparation will then depend on application:

- **chemical fixation**, resin embedding, thin sectioning.
- **fast freezing**, cryo-substitution, resin embedding and thin section deposited on metal or silicon substrate.

Biological sample preparation

less redistribution of highly diffusable trace metals !

Chemical fixation	Cryo fixation
Glutaraldehyde Formaldehyde Osmium tetroxide	**high pressure freezer** tissues (up to 6 mm diameter, 200 µm thick)

Dehydration	Dehydration
Solvent baths (acetone or ethanol/water) with increasing solvent concentrations	Cryo-substitution lyophilization

Resin embedding	Resin embedding
Solvent baths with increasing resin concentrations	

Ultramicrotomy

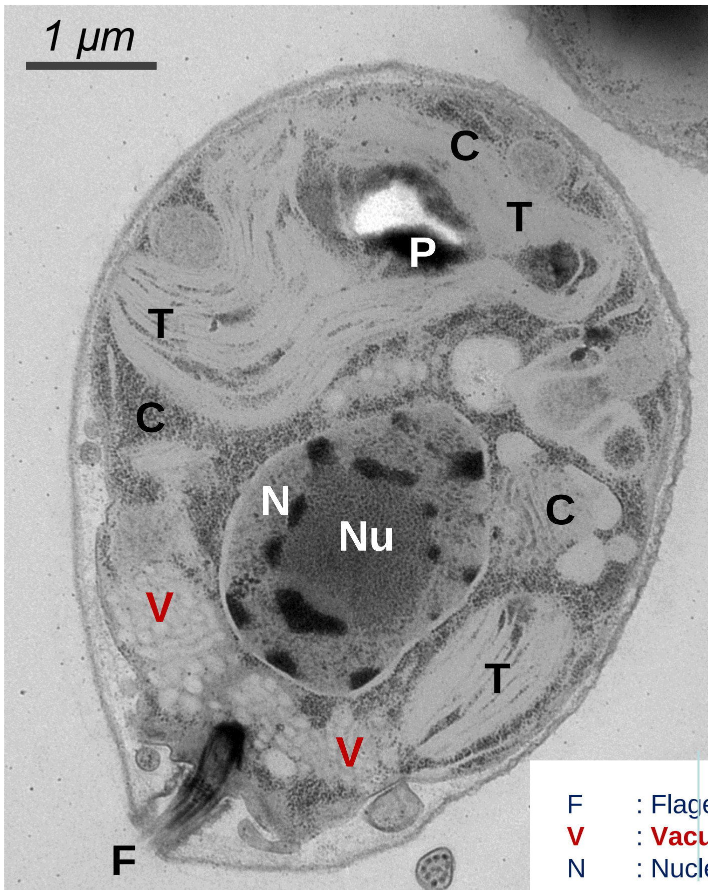
300-400 nm sections

Equipment at Bordeaux Imaging Center

Application to a model organism:
Chlamydomonas reinhardtii cells (unicellular green algae)

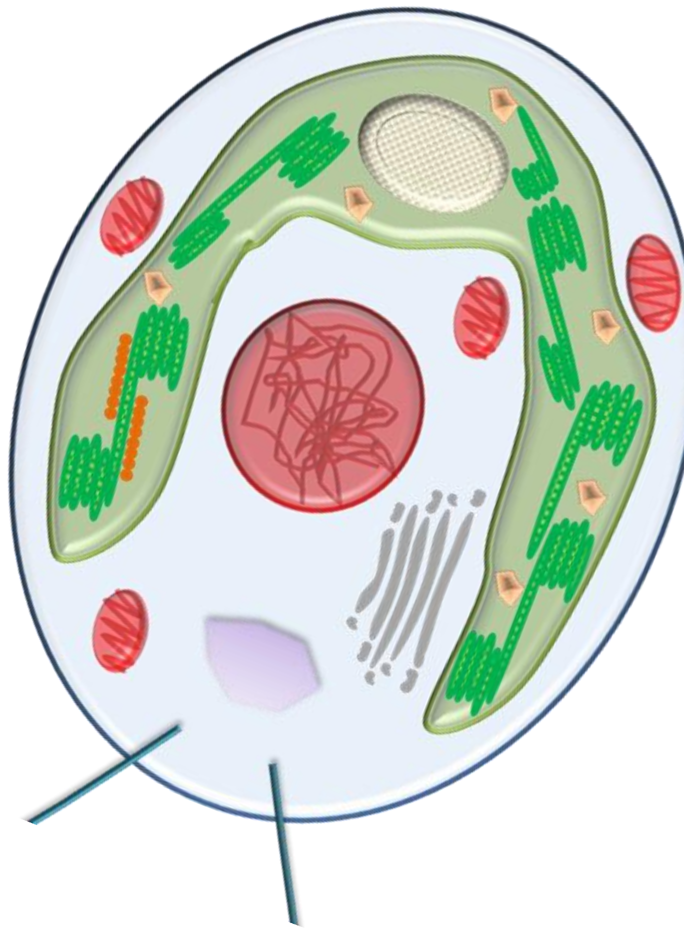
TEM analysis (70 nm thin section)

resolution down to 1 nm



F : Flagella
V : Vacuoles
N : Nucleus
Nu : Nucleolus
C : Chloroplast
T : Thylakoid
P : Pyrenoid

Comparison with schematic view



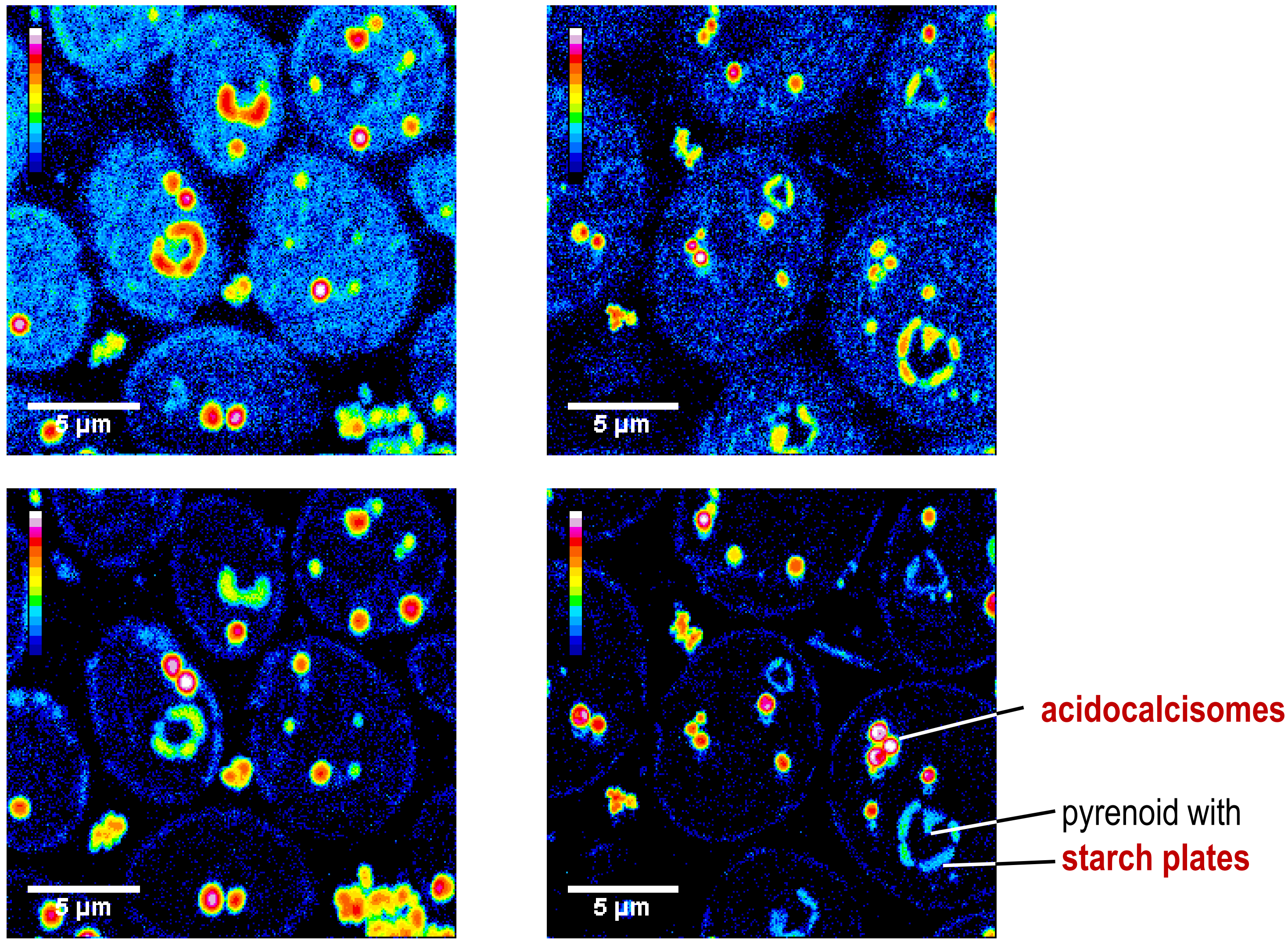
NanoSIMS analysis of *Chlamydomonas reinhardtii* cells

Comparison conventional Duoplasmatron O^- ion source and novel O^- ion source

Duoplasmatron O- ion source | **New O^- ion source prototype**

^{23}Na

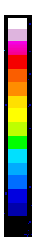
^{40}Ca



300 nm
thin sections

relative intensity:

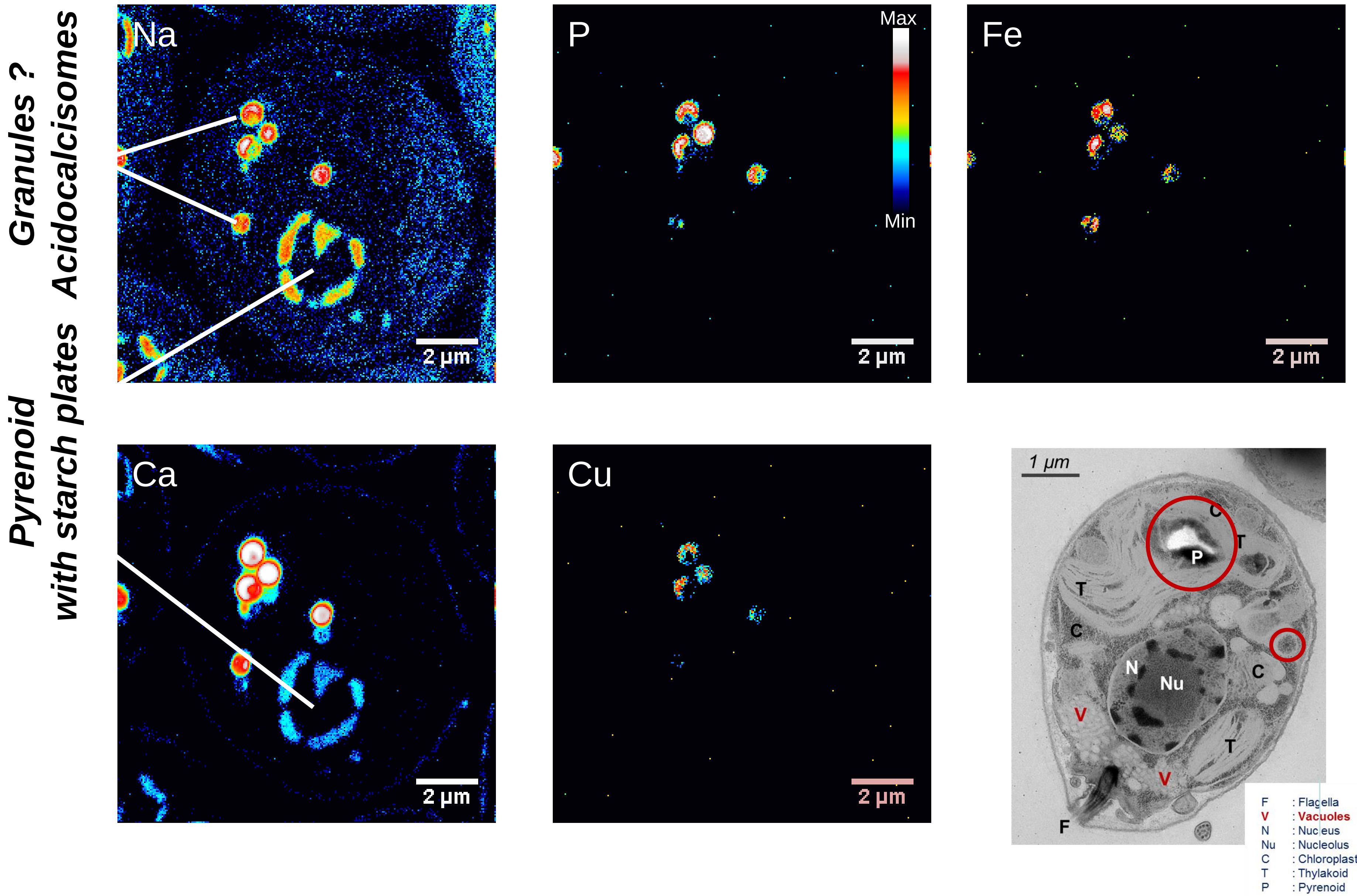
Max



Min

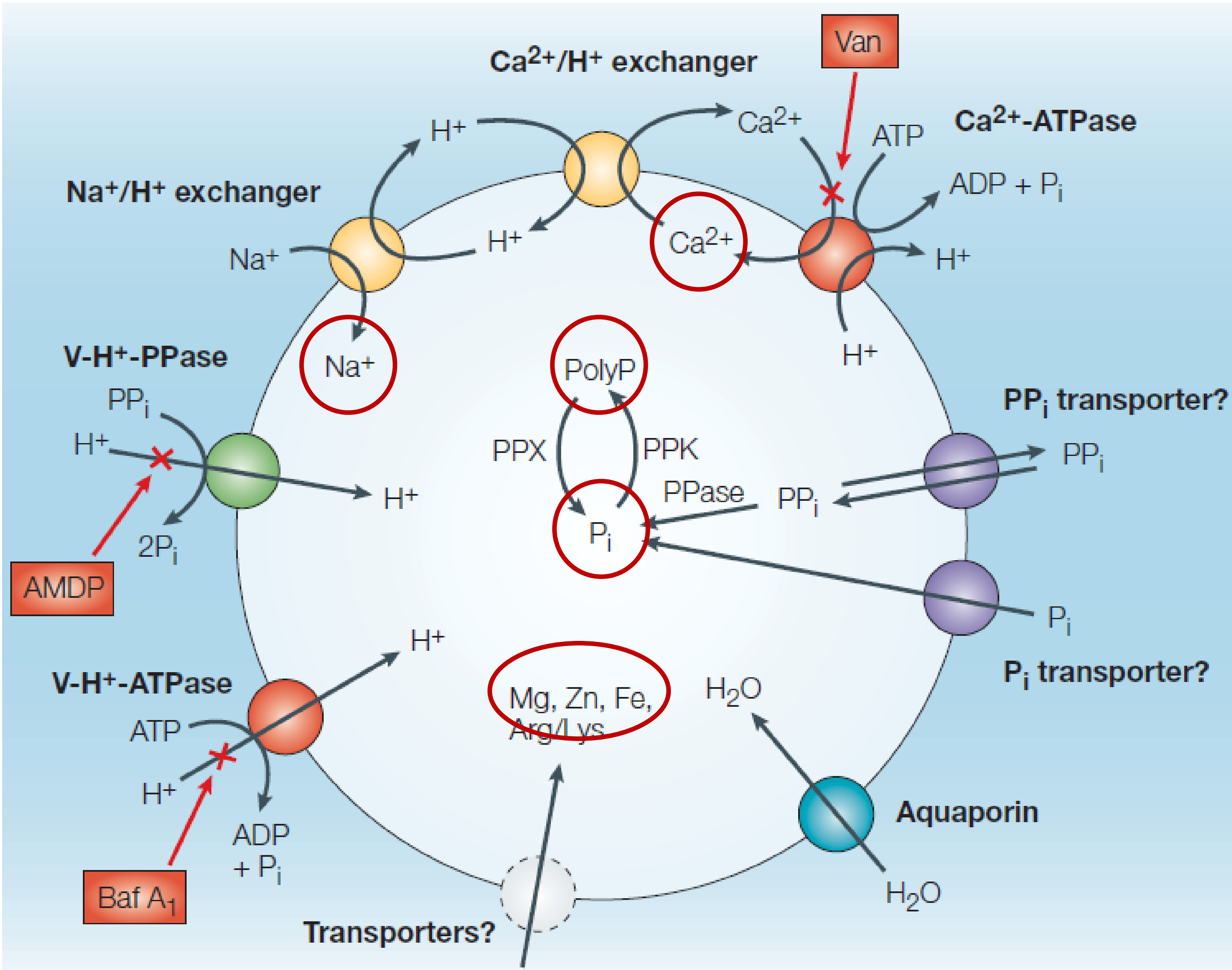
20 x 20 µm
12 min
256x256 pixel
1 plane

Subcellular element imaging by NanoSIMS (new ion source)



Single cell imaging: 12 x 12 µm, 22 min, 512x512 pixel, 2 planes

Scheme of a Acidocalcisome



R. Docampo, W. de Souza, K. Miranda, P. Rohloff, S. N. J. Moreno, *Nature Reviews* 2005, 3, 251-261

Biological applications

Use of the conventional cesium primary ion source for isotopic tracer experiments (C-13): Investigation of physiological processes under Cd stress

Parallel TEM/X-EDS experiments

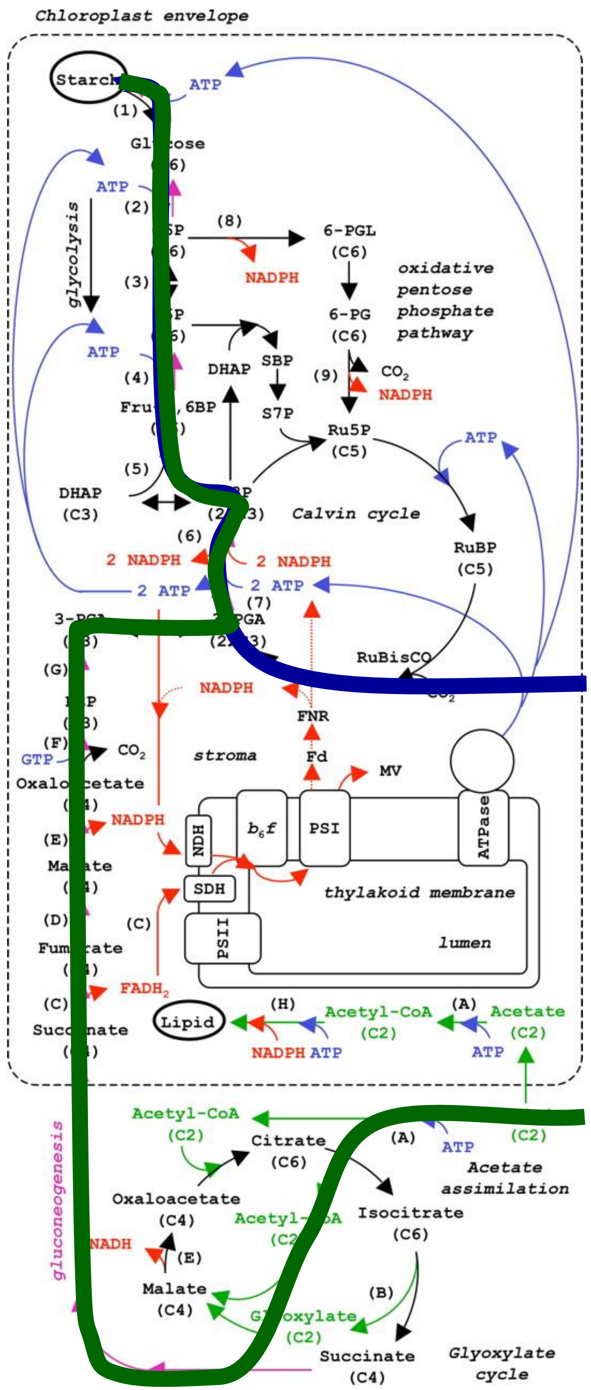
starch

Investigation of the CO_2 fixation Under Cd stress

NanoSIMS with <u>Cs source</u>
TEM/X-EDS

Simplified schematic drawing of metabolic pathways in Chlamydomonas reinhardtii

Xenie Johnson, and Jean Alric J. Biol. Chem. 2012;287:26445-26452



Enzyme RuBisCo

CO_2 : ^{12}C

acetate : ^{13}C

jbc

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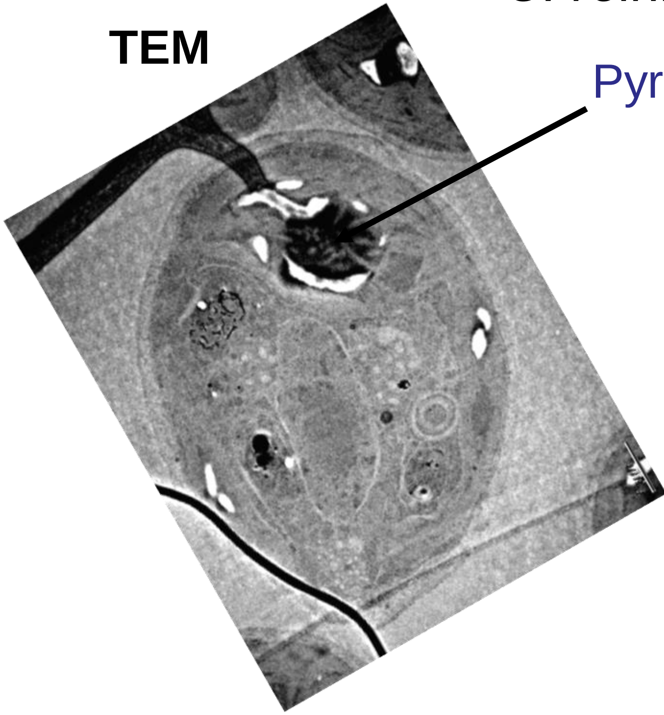
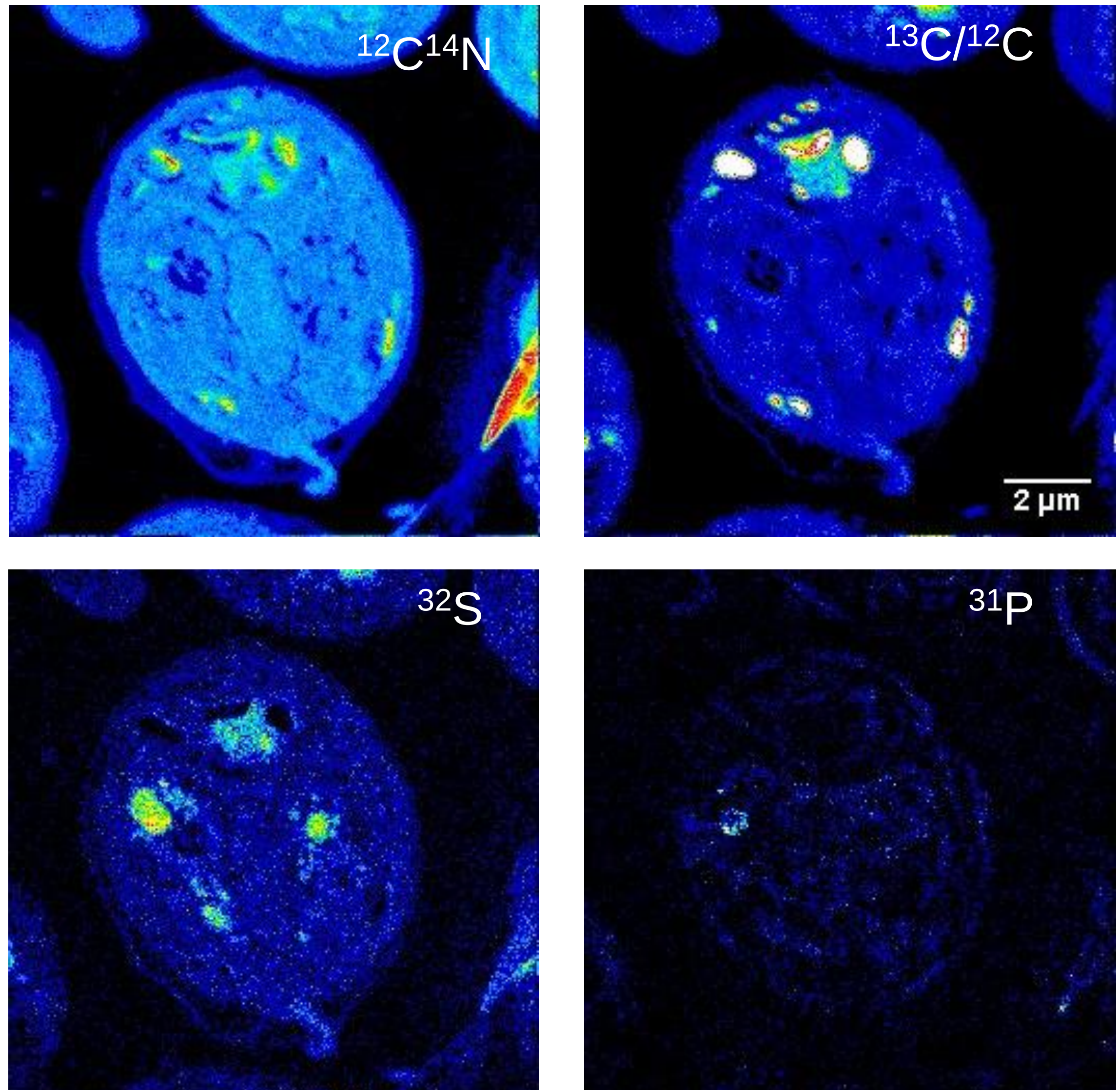
C. reinhardtii wt in mTAP medium 70 µM Cd

TEM

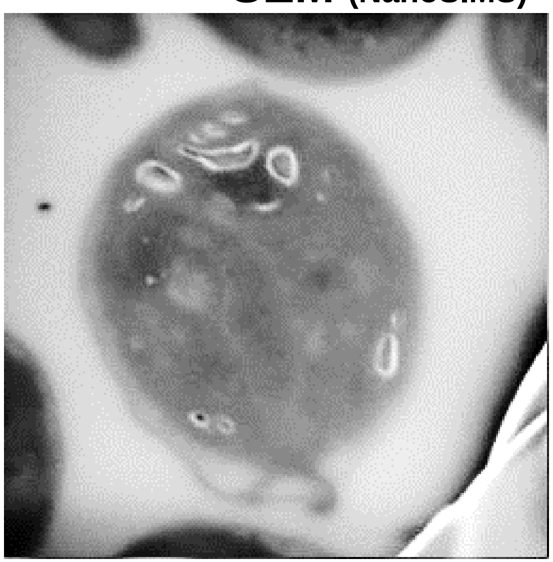


Pyrenoid: accumulation of RuBisCo

NanoSIMS

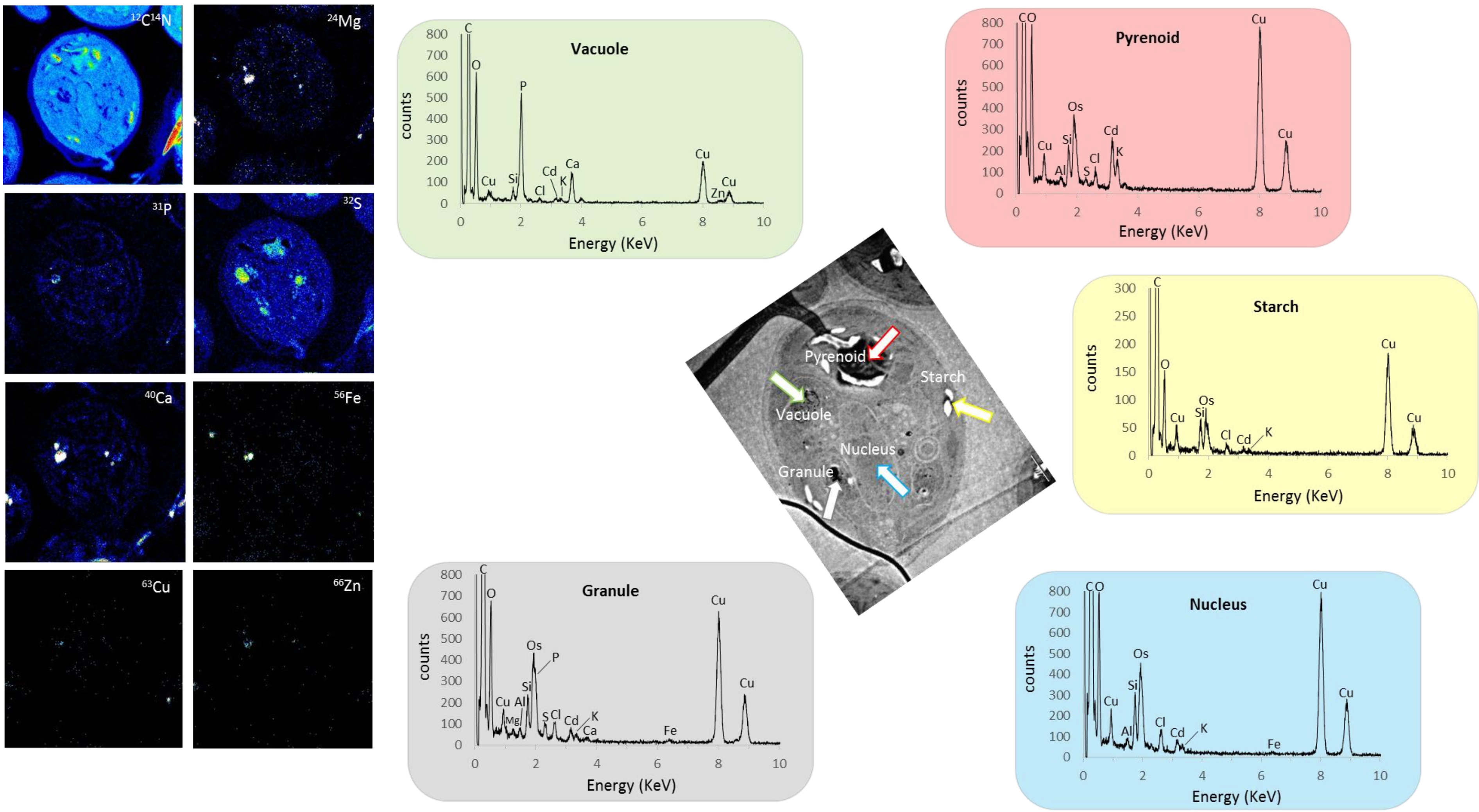


SEM (NanoSIMS)



NanoSIMS

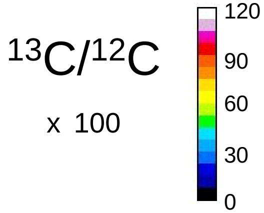
TEM/X-EDS



C. reinhardtii

wt in mTAP medium

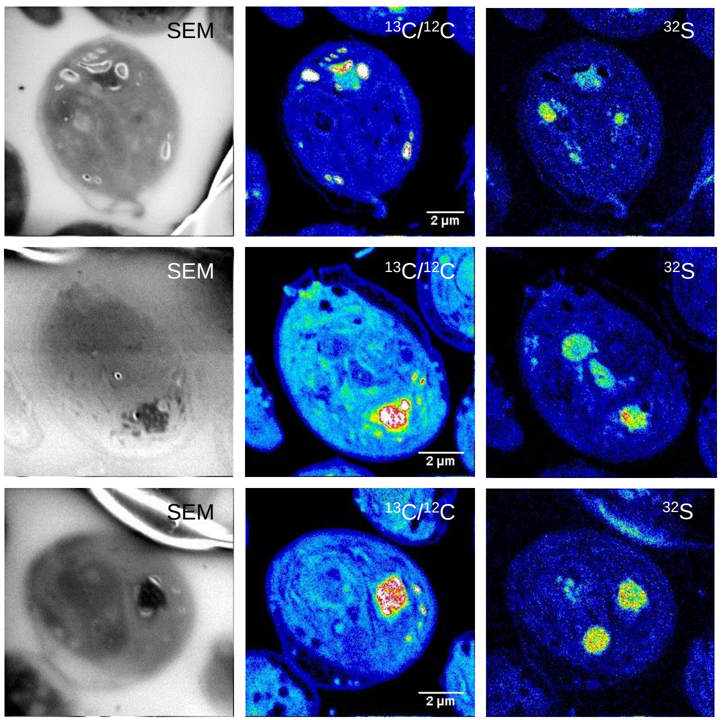
70 µM Cd

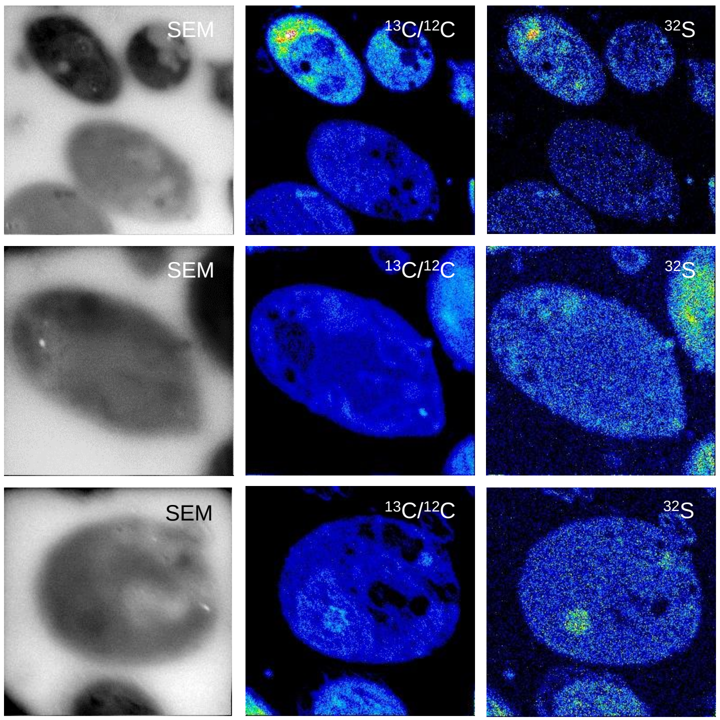


C. reinhardtii

wt in mTAP medium

control

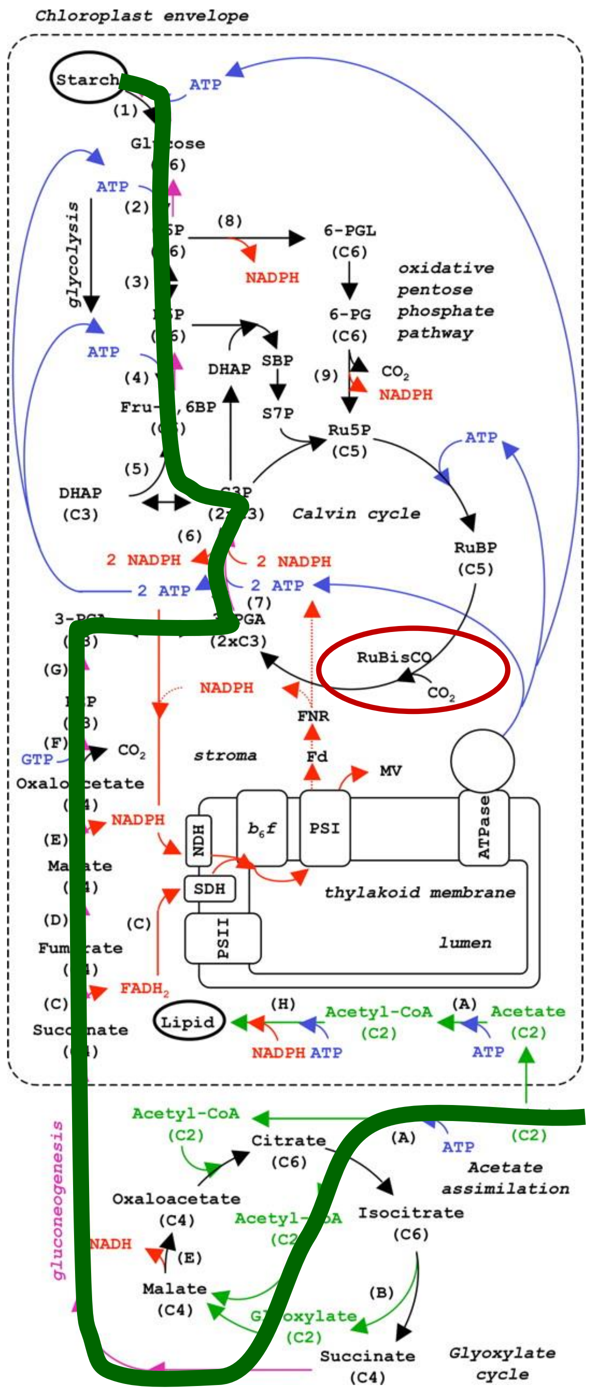




Simplified schematic drawing of metabolic pathways in Chlamydomonas reinhardtii

Xenie Johnson, and Jean Alric J. Biol. Chem. 2012;287:26445-26452

starch



RuBisCo blocked by Cd

~~CO_2~~ : ^{12}C

acetate : ^{13}C

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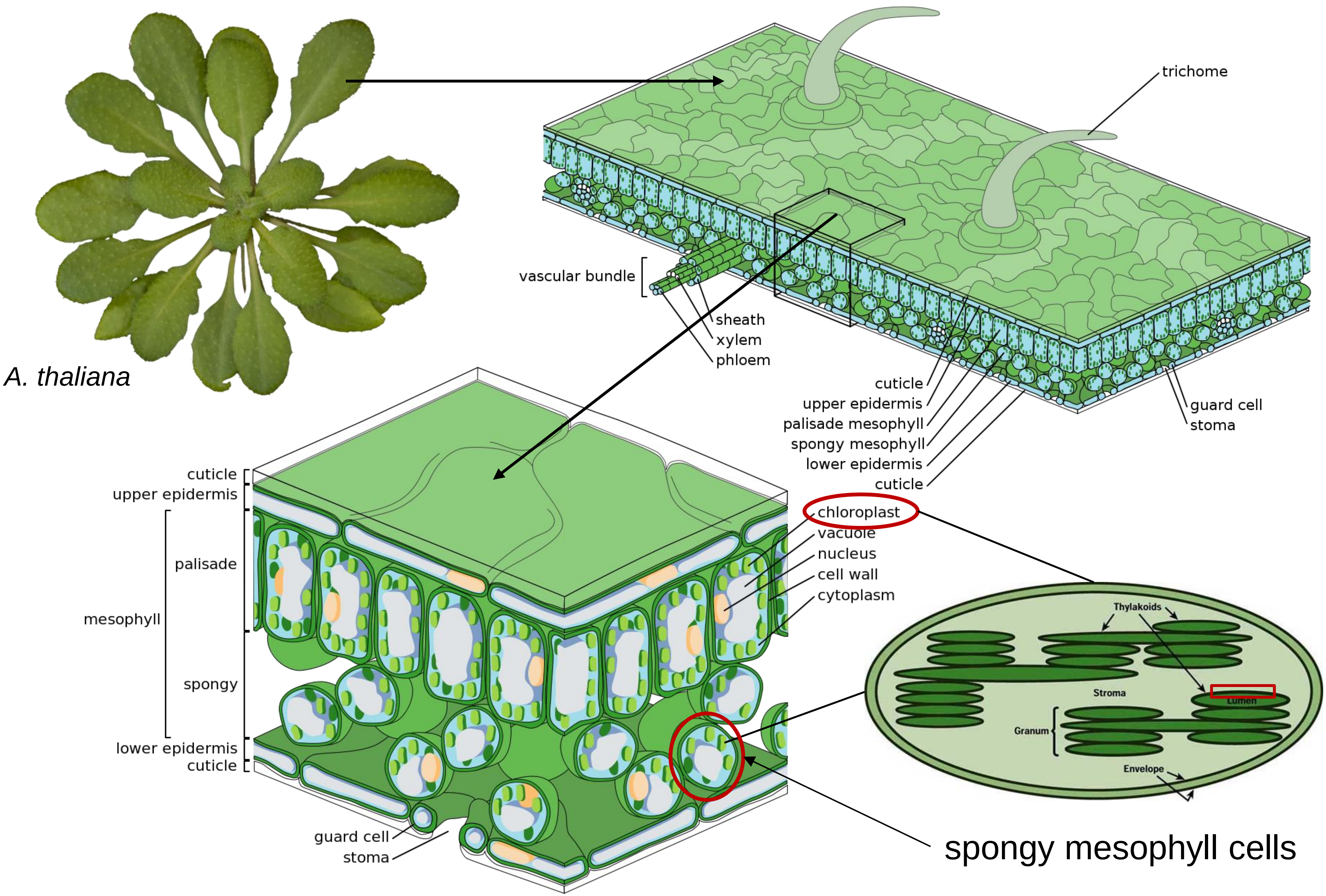
jbc

Biological applications

Use of the novel oxygen primary ion source for the localization of major (**Na, Ca, Mg**) and trace (**Fe, Cu, Mn, Zn**) metals Involved in physiological processes in plant cells

Insight into photosynthesis

Subcellular localisation of metals in *Arabidopsis thaliana* leaf cells



Essential (trace) metals involved in photosynthesis

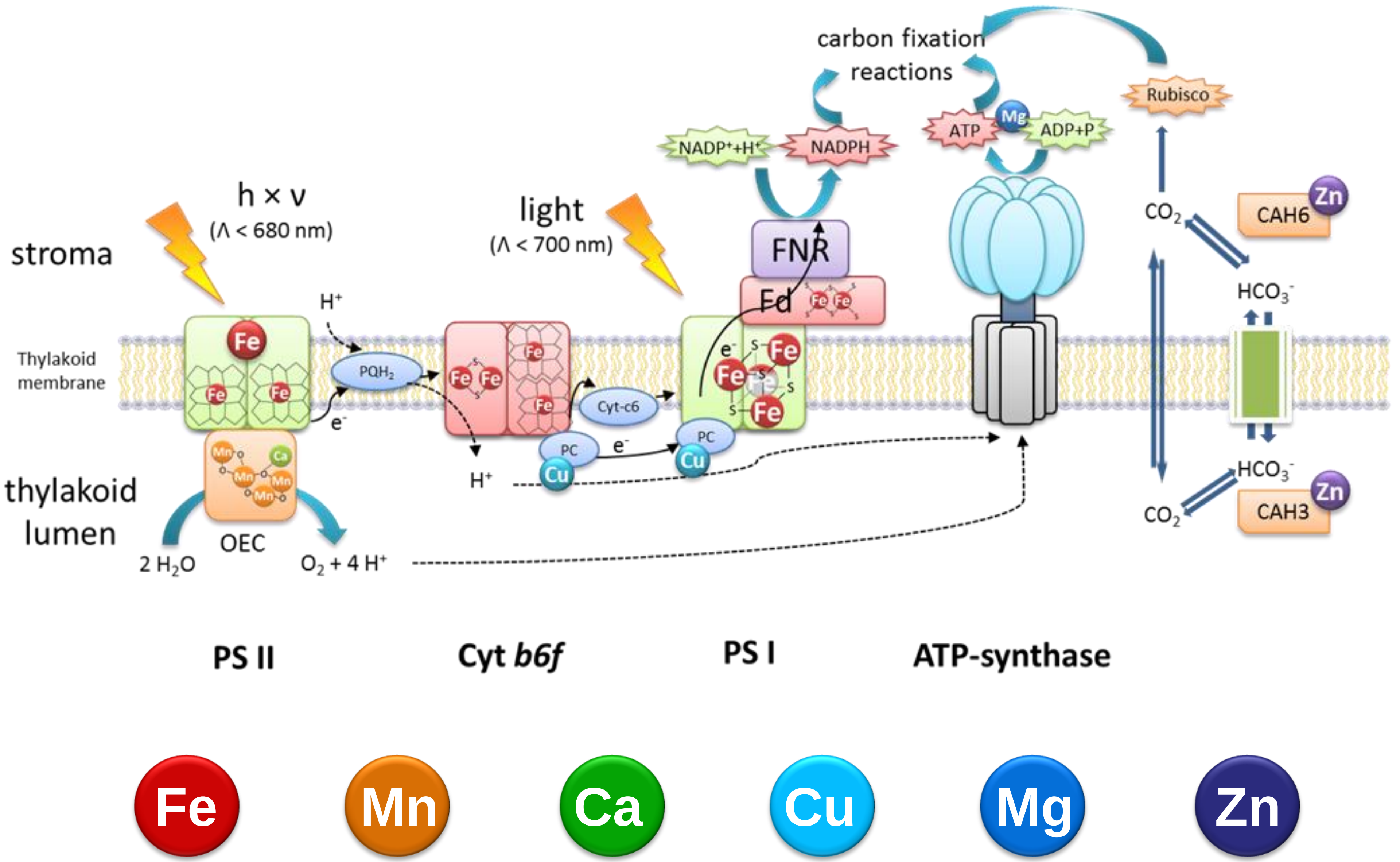
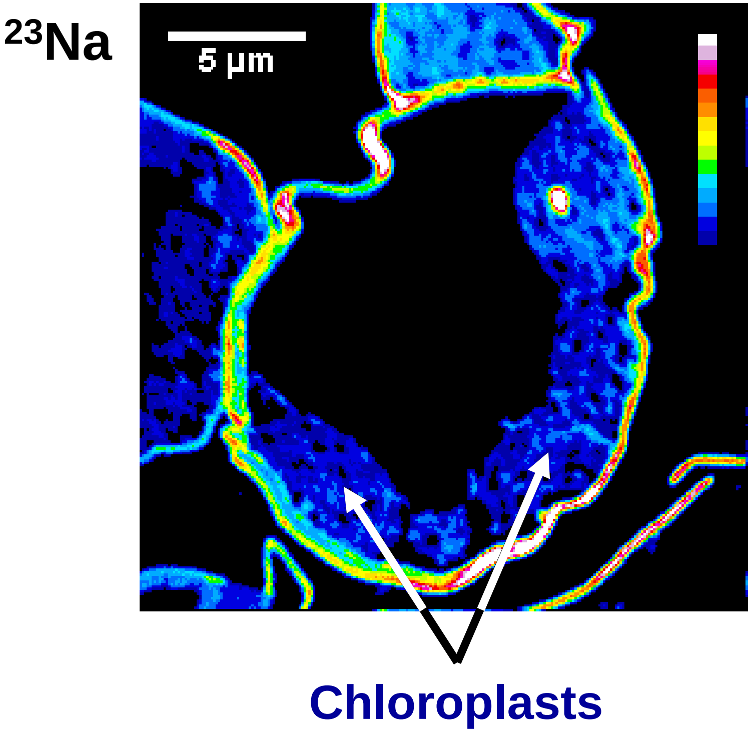
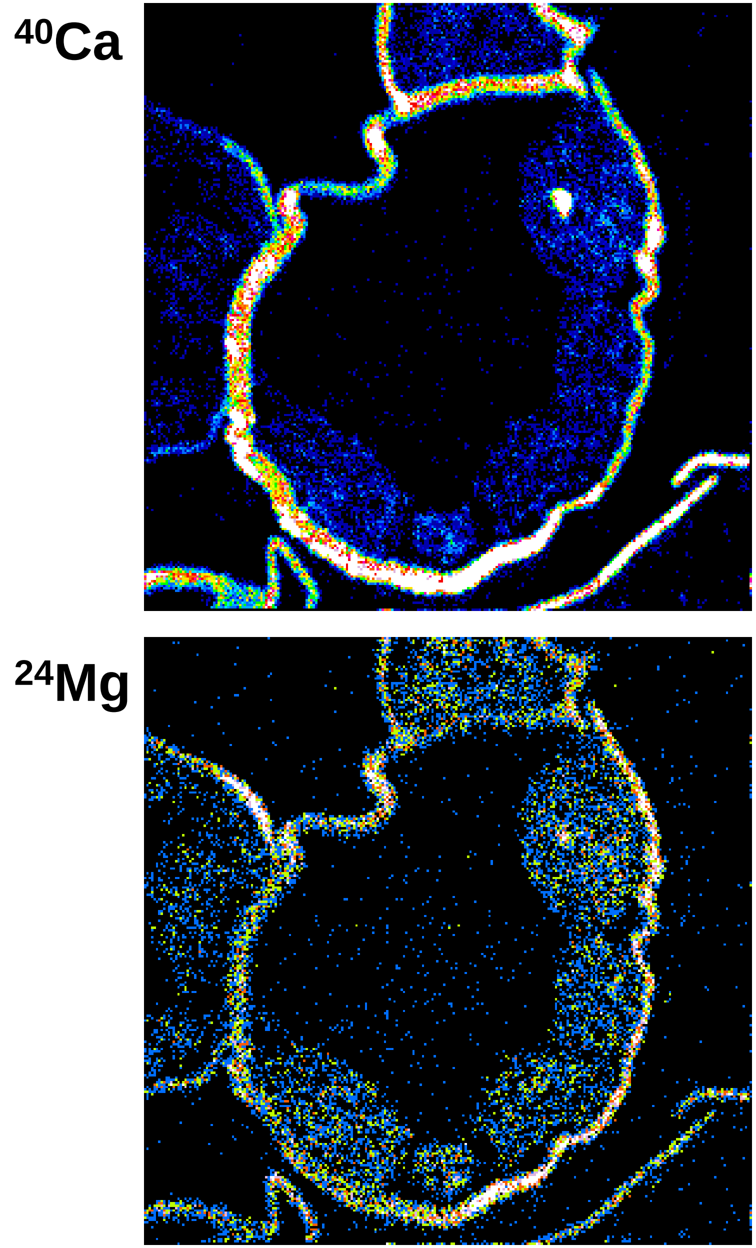


Image of a whole cell: sodium, calcium and magnesium imaging



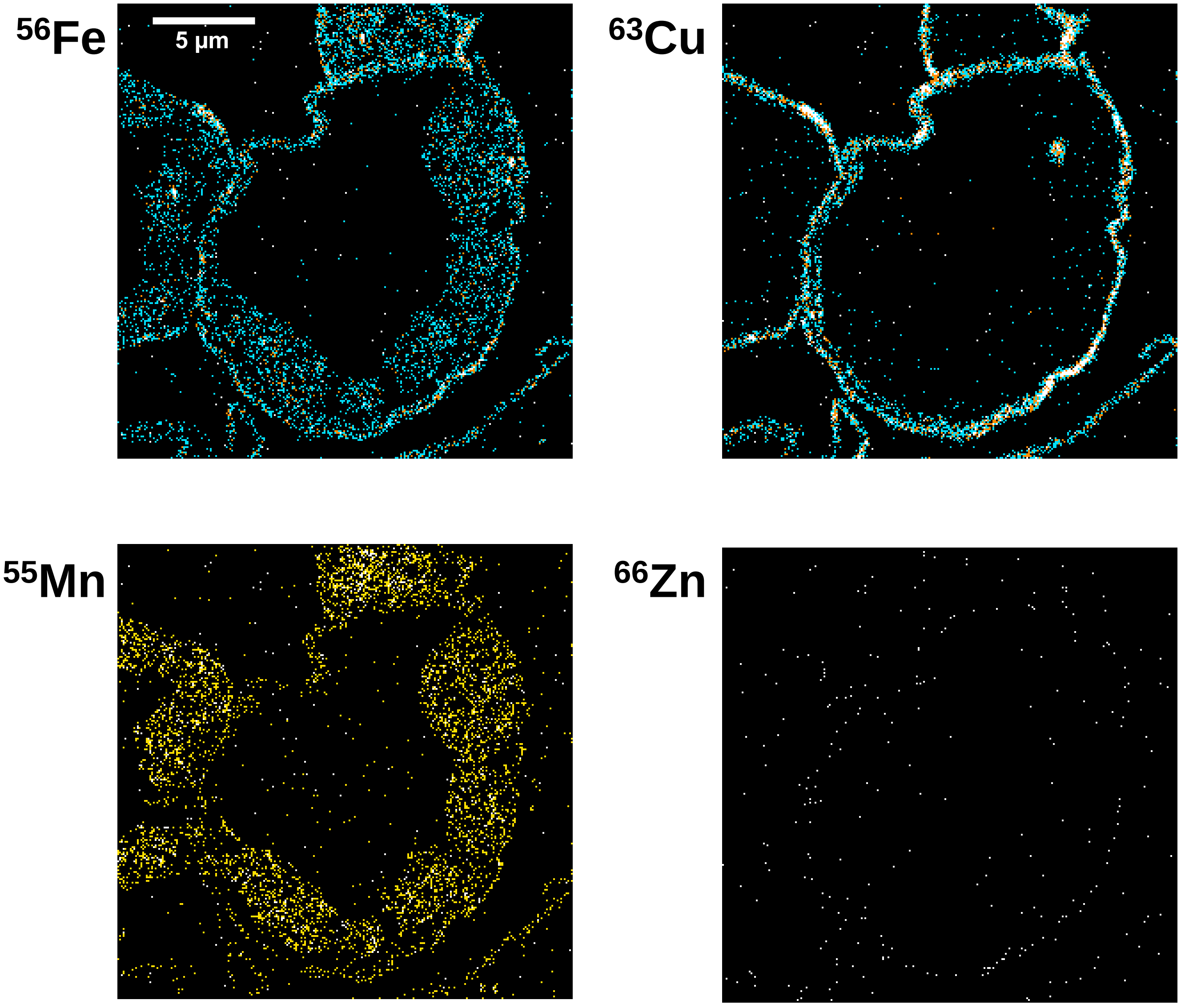


256 x **256** pixel
22 x 22 µm

1 plane

Total acquisition time: **5.5 min**

Trace element imaging



256 x **256** pixel
22 x 22 µm

Overlay of **12** planes
(single images)

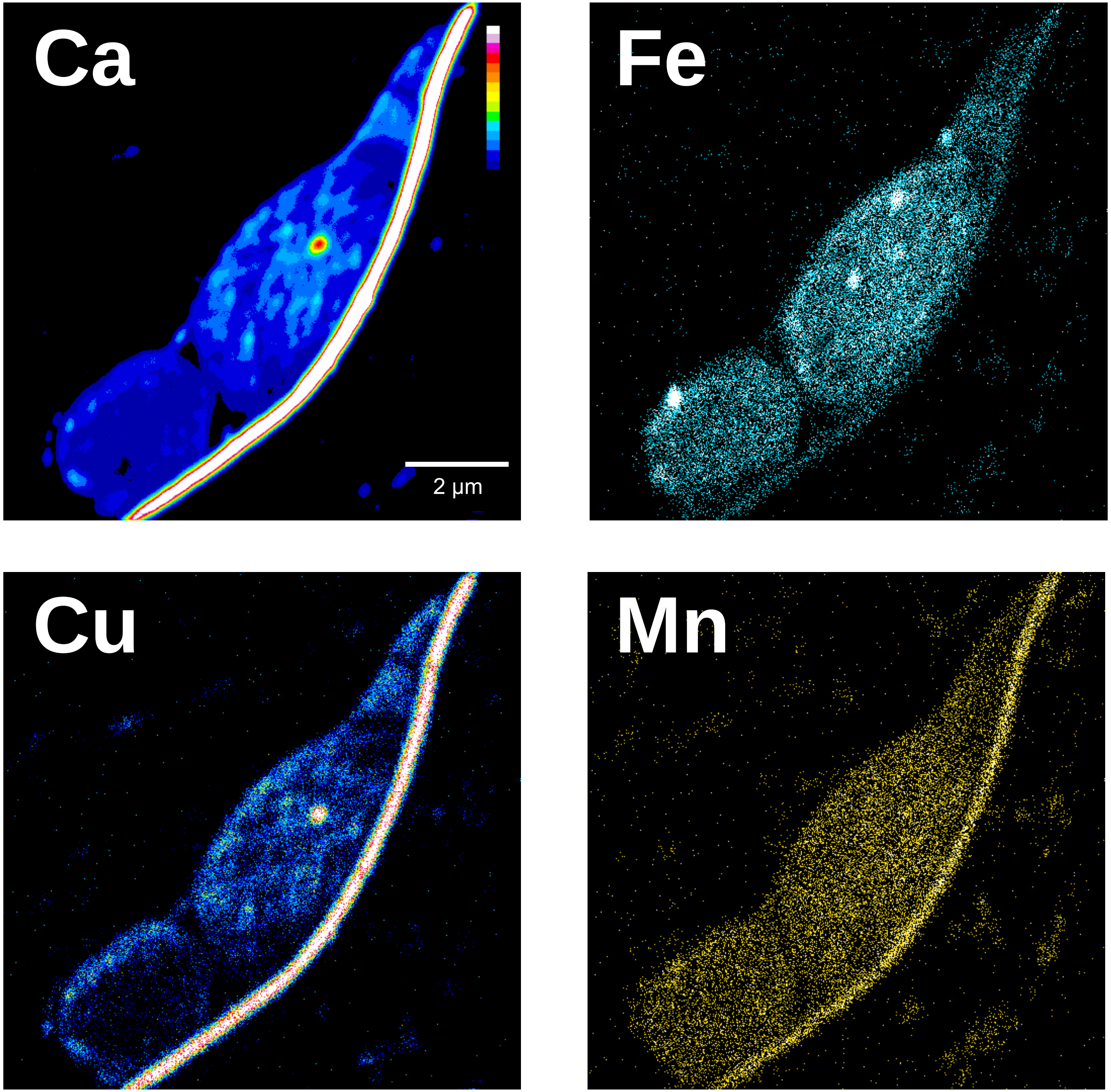
Total acquisition
time: **1.5h**
(7.6 min/plane)

Insight into the chloroplast

512 x **512** pixel
10 x 10 µm

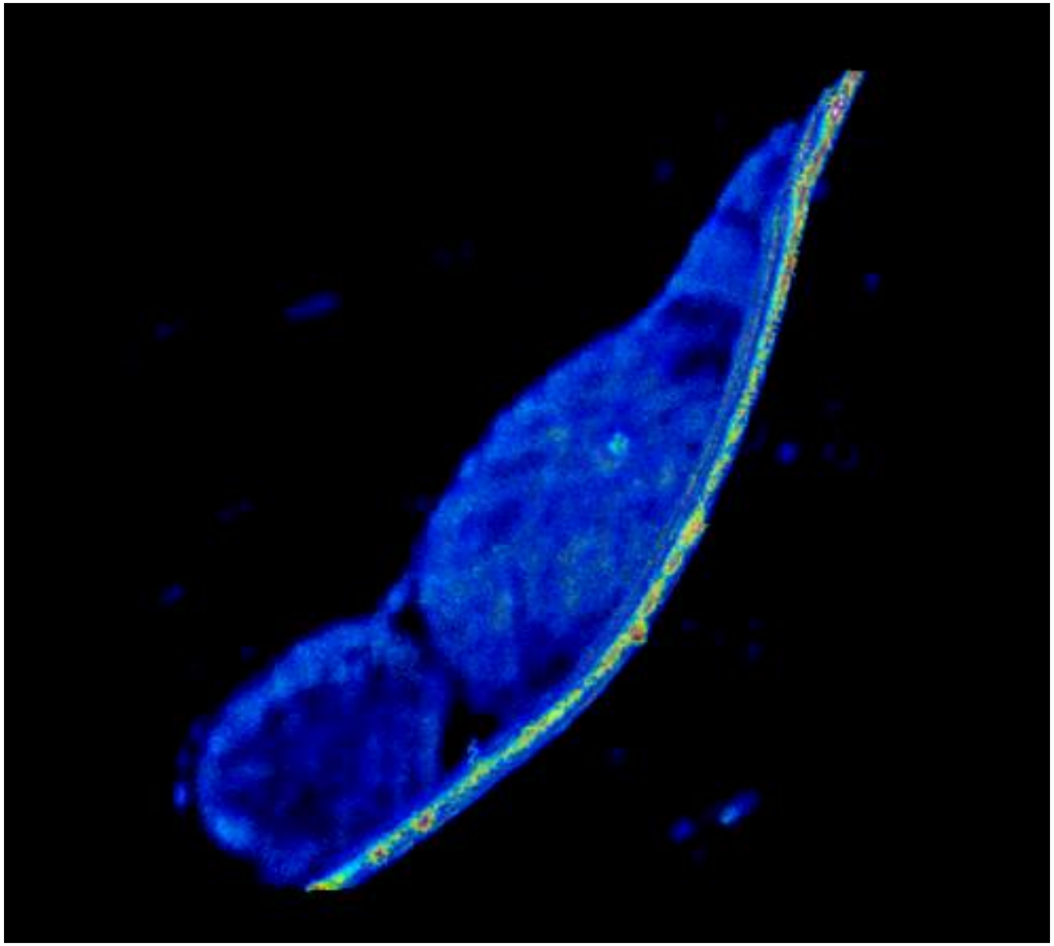
Overlay of **30** planes
(single images)

Total acquisition
time: **11h**
(21.8 min/plane)



3D reconstruction of 30 successive planes :

^{40}Ca



Conclusions

NanoSIMS offers many applications in different scientific fields :
Microbiology, Cell Biology, Environment
but also Material Sciences, Cosmology, Geochemistry :

- Surface analysis by imaging, element quantification, and isotope analysis
- High spatial resolution: 50 x 50 nm in *2D*
  50 x 50 x 10 nm in *3D*
- Parallel detection up to 7 masses
- High sensibility
- High mass resolution

A **novel Oxygen primary ion source** shows high stability and high resolution and allows a parallel sensitive detection of biologically relevant major and trace elements at subcellular level.

Acknowledgements

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Marie-Pierre Isaure Lecturer

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Ivo Bertalan (plant biochemistry)
Gerd-J. Krauss (plant biochemistry)
Martin Hertzberg (biochemistry)
Gerd Hause (sample preparation)

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Project: **ANR-11-EQPX-0027 – Mass Spectrometry Center MARSS**


Thank you for
your attention !