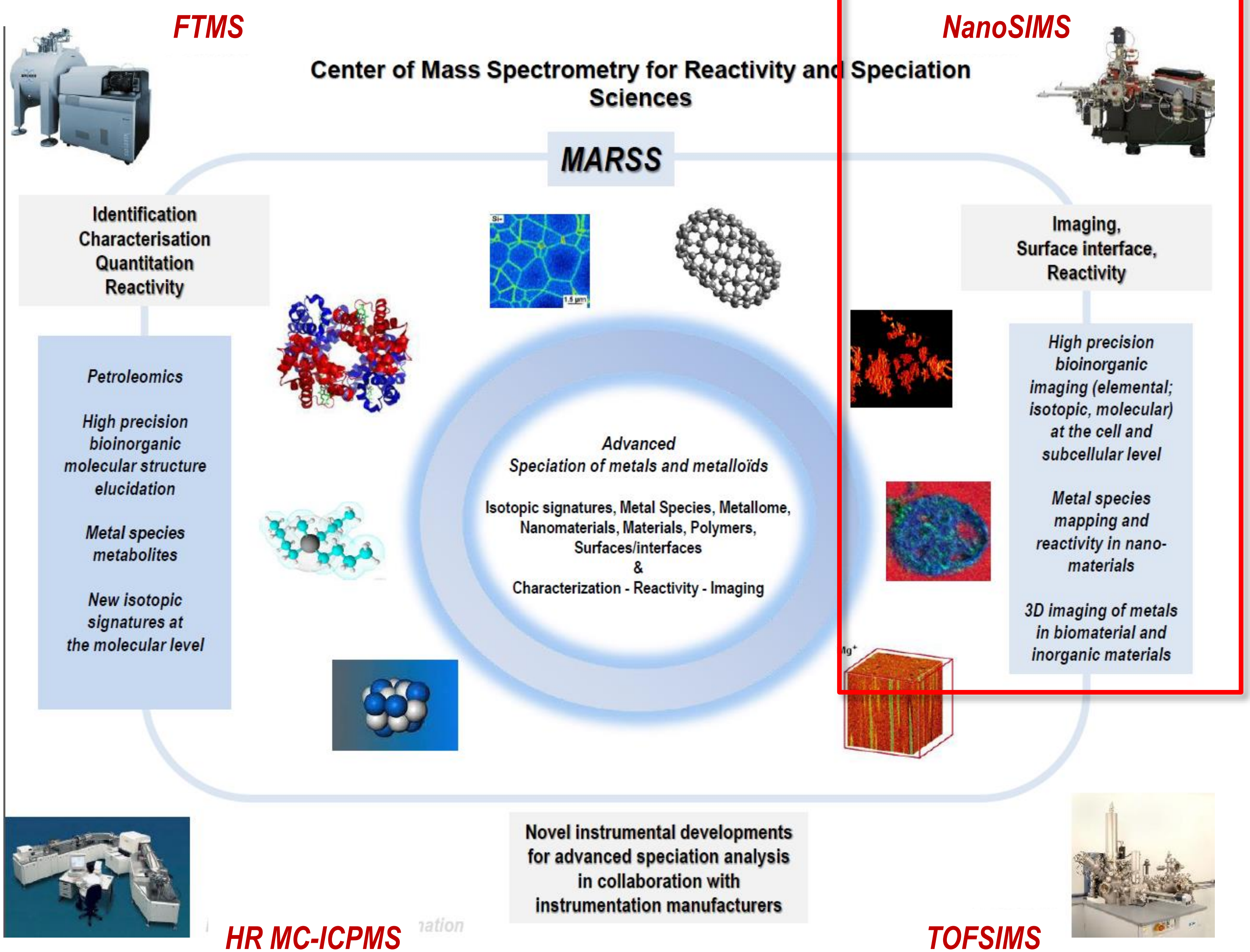
A RF plasma oxygen ion source on NanoSIMS for subcellular trace element detection

Dirk Schaumlöffel[1], Julien Malherbe[1], Étienne Gontier[2]
François Hillion[3], François Horréard[3], Dirk Dobritzsch[4]

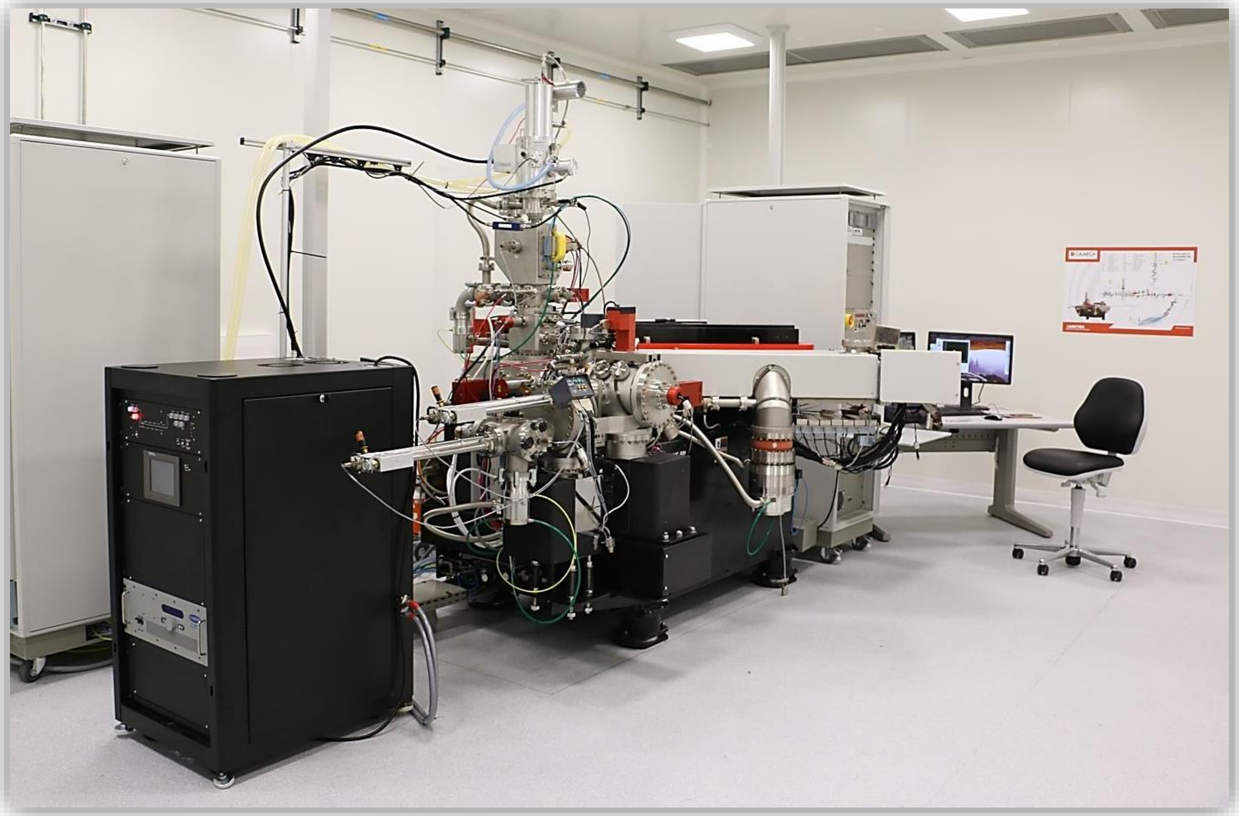
[1]**Université de Pau et des Pays de l'Adour / CNRS**
Institut des Sciences Analytiques et de Physico-Chimie
pour l'Environnement et les Matériaux, UMR 5254 IPREM/LCABIE, Pau, France
[2]**Bordeaux Imaging Center,** Pôle d'imagerie électronique, Bordeaux, France
[3]**CAMECA,** 29 Quai des Grésillons, Gennevilliers, France
[4]**Martin-Luther-Universität Halle-Wittenberg**
Institut für Biochemie und Biotechnologie, Abteilung Pflanzenbiochemie, Halle (Saale), Germany

The 7th International NanoSIMS user meeting
"NanoSIMS & correlative microscopy: exploring physical and biochemical boundaries"
Leipzig, Germany, 22-24th of August 2017

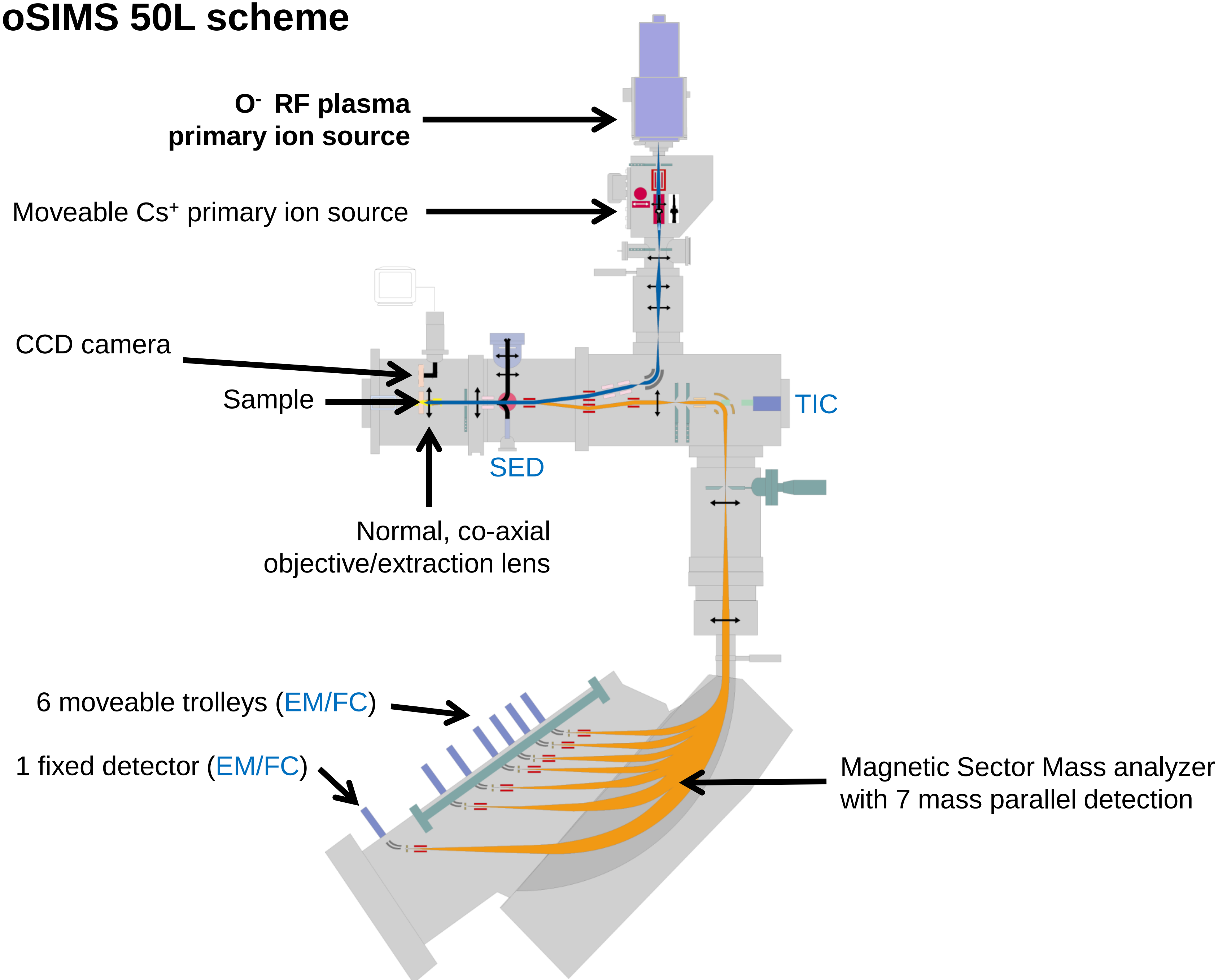
Equipex MARSS project



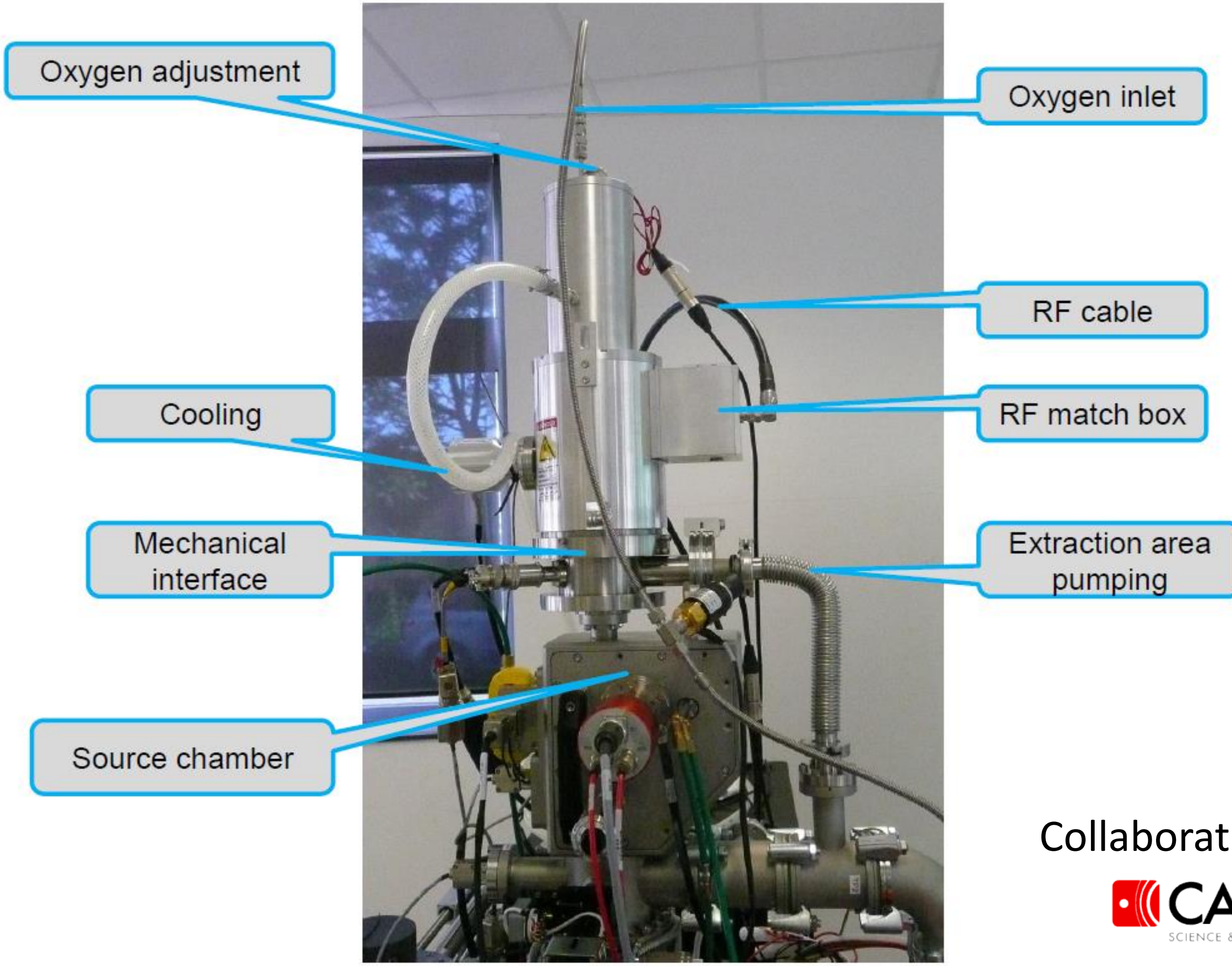
NanoSIMS delivery and installation in Pau (March – June 2017)



NanoSIMS 50L scheme



A new RF plasma O^- primary ion source on NanoSIMS

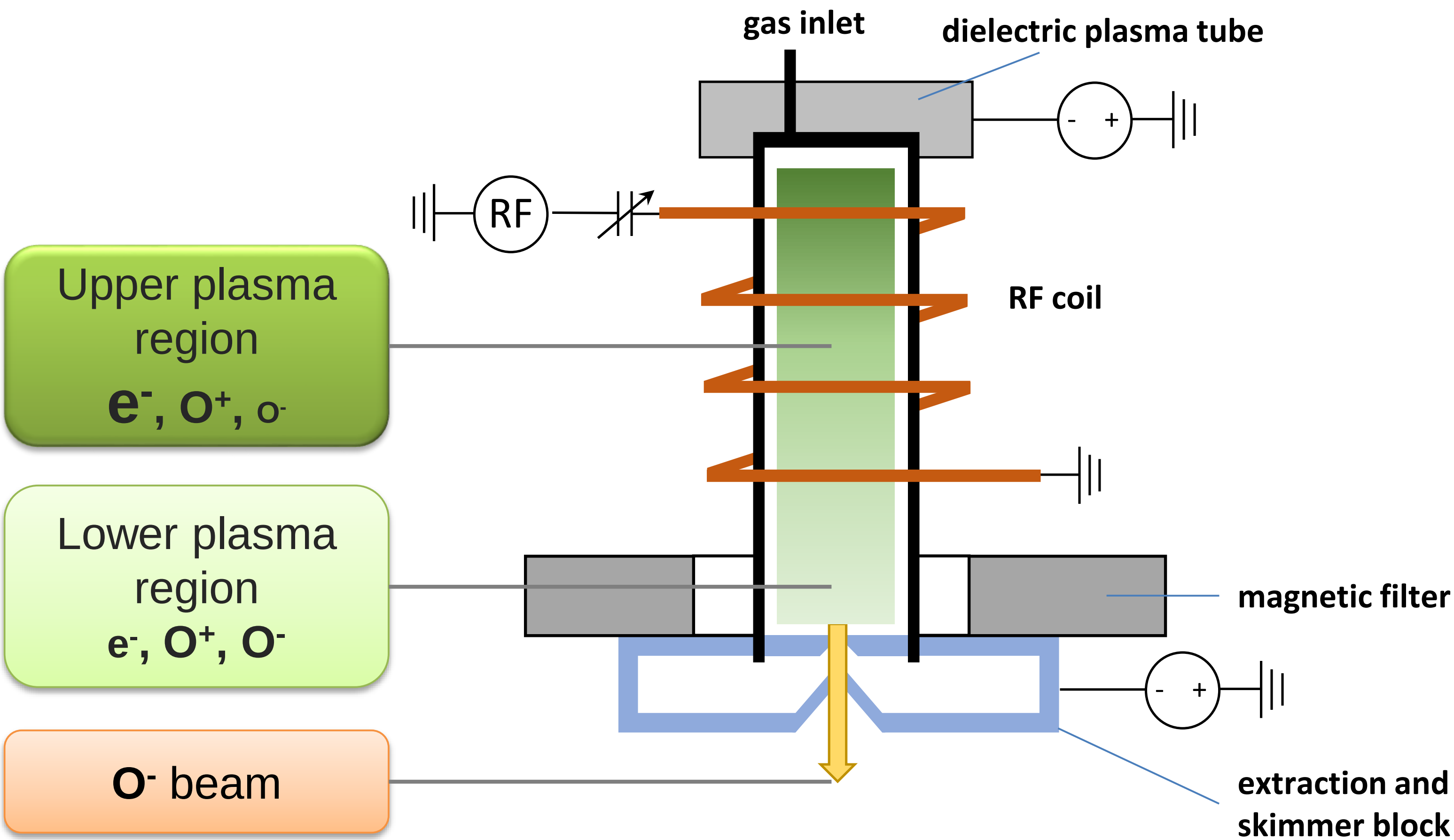


Collaboration with

CAMECA
SCIENCE & METROLOGY SOLUTIONS

Hyperion™ source manufactured by Oregon Physics (Hillsboro, OR)

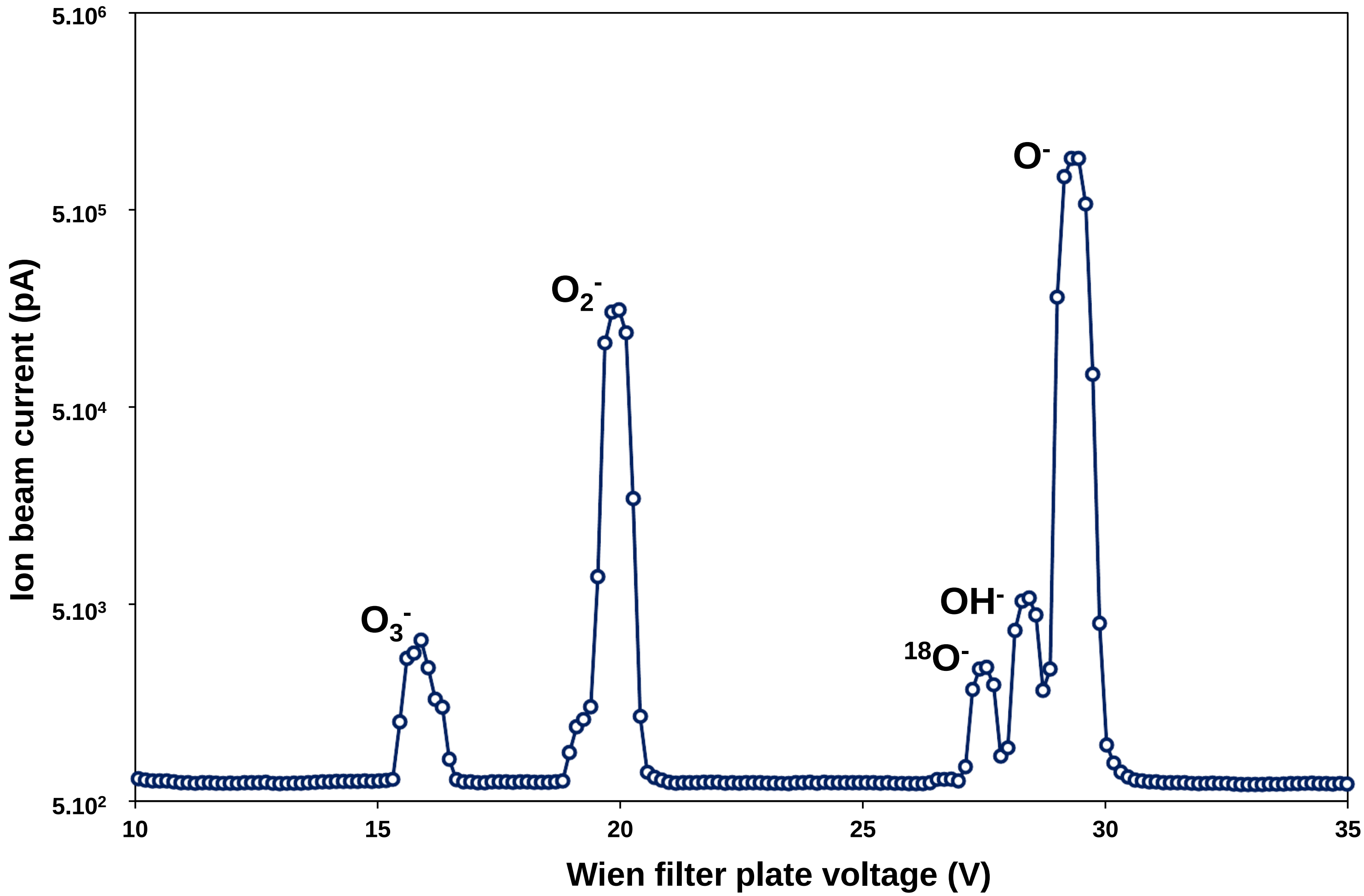
Schematic view of the RF plasma O^- primary ion source



Source diameter : 70 - 80 µm *(manufacturer specification 35-50 µm)*

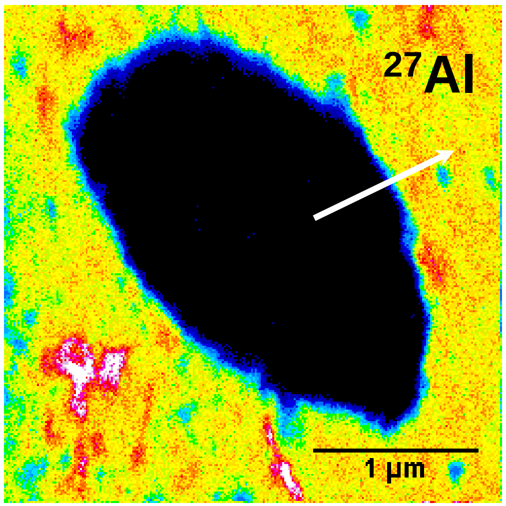
Brightness: ~ 100 mA×cm^{-2}×sr^{-1} at 8 kV

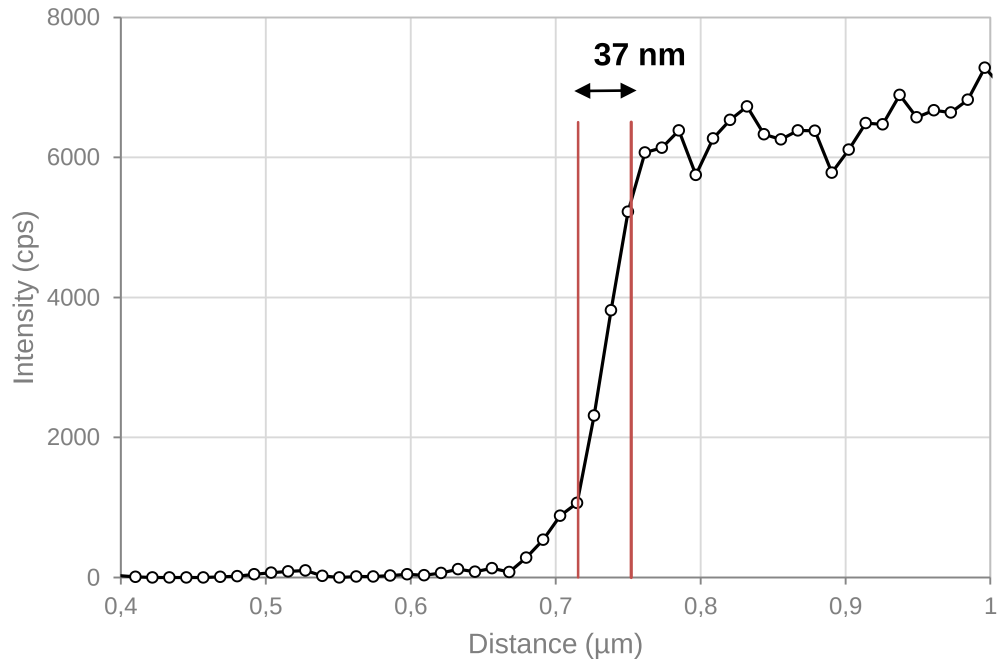
Oxygen ion distribution of the primary beam using a Wien filter located after the source



O^- ions represent approximately 88% of the distribution

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



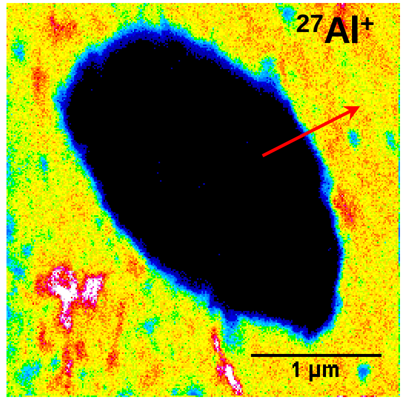


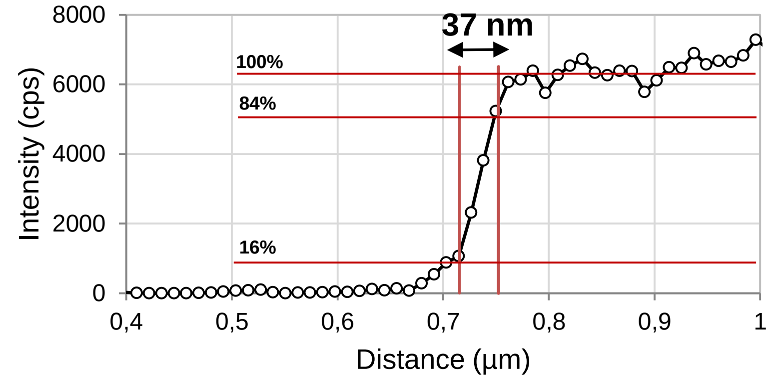
Si oxide grain sample over Al substrate
Image size: 3 x 3 µm
Probe intensity: 0.15 pA

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

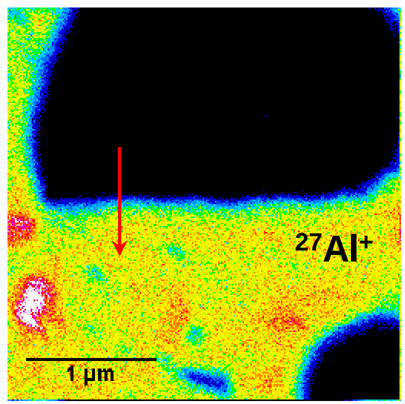
Comparison with Duoplasmatron and Cs primary ion sources

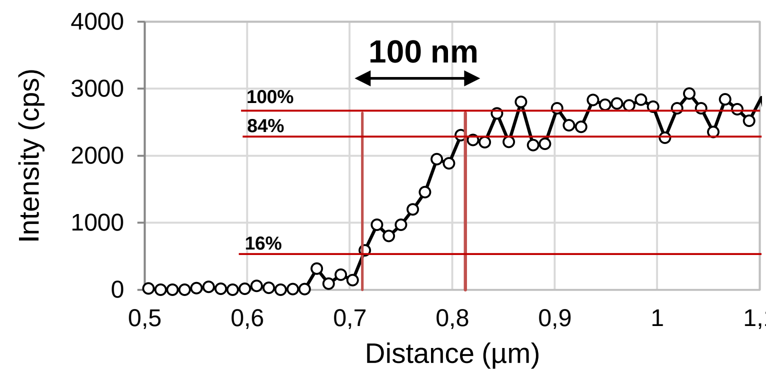
RF plasma source, 0.15pA



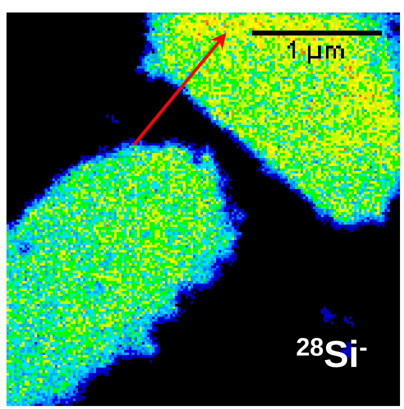


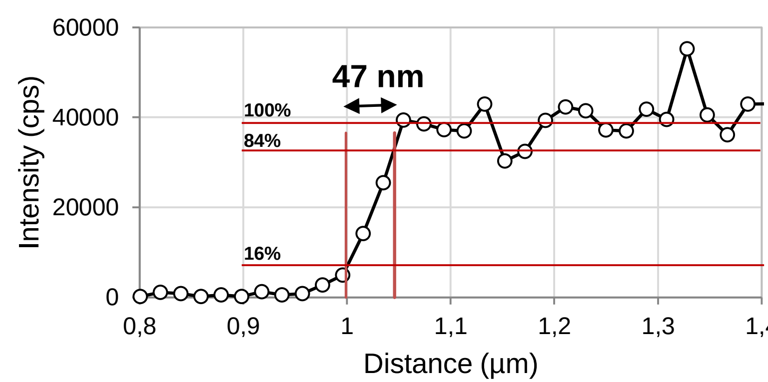
Duoplasmatron Source, 0.1pA



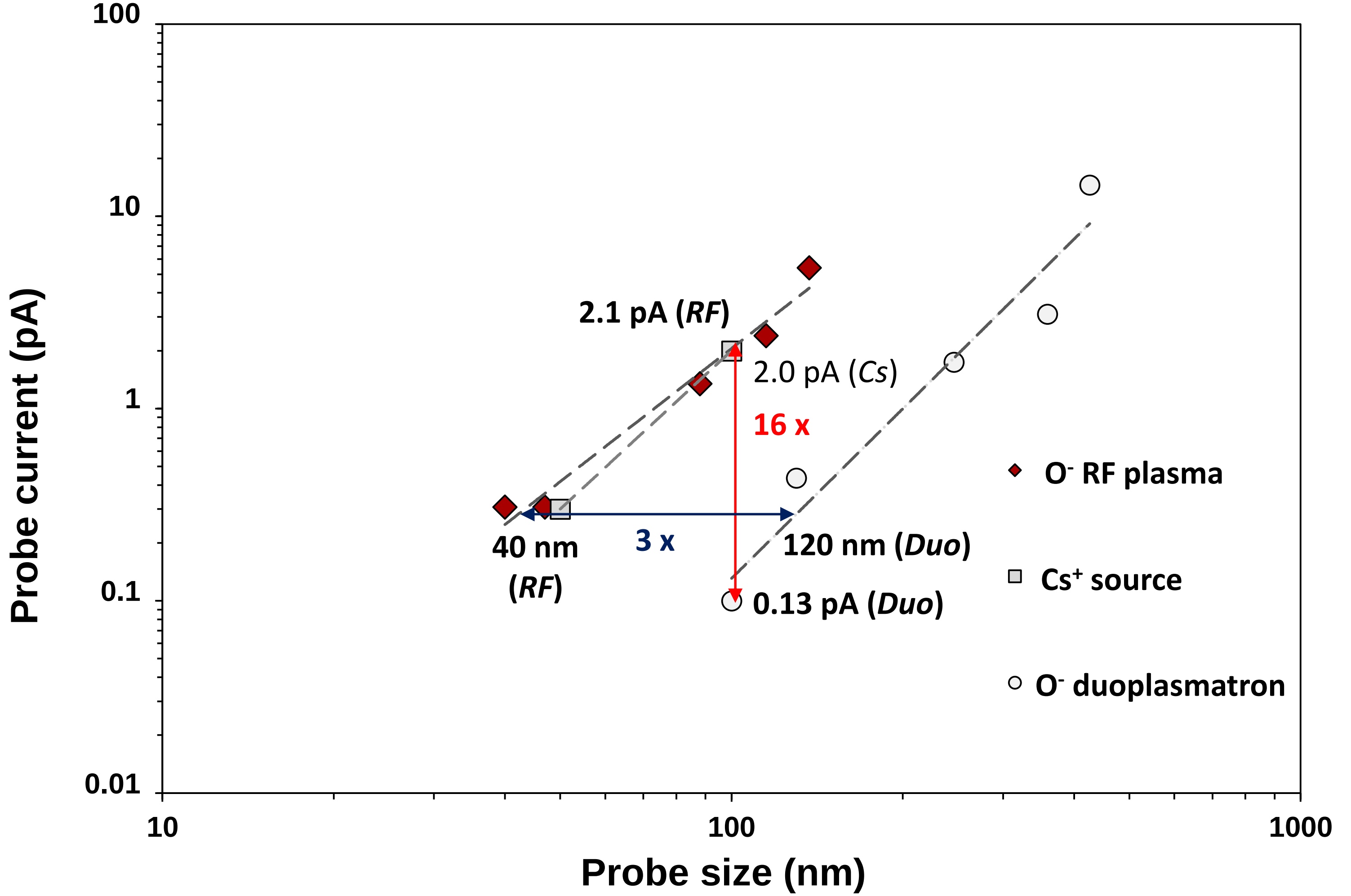


Cs^{+} source, 0.17pA





Comparison of the sample current density for the Cs$^+$, O$^-$ duoplasmatron and O$^-$ RF sources

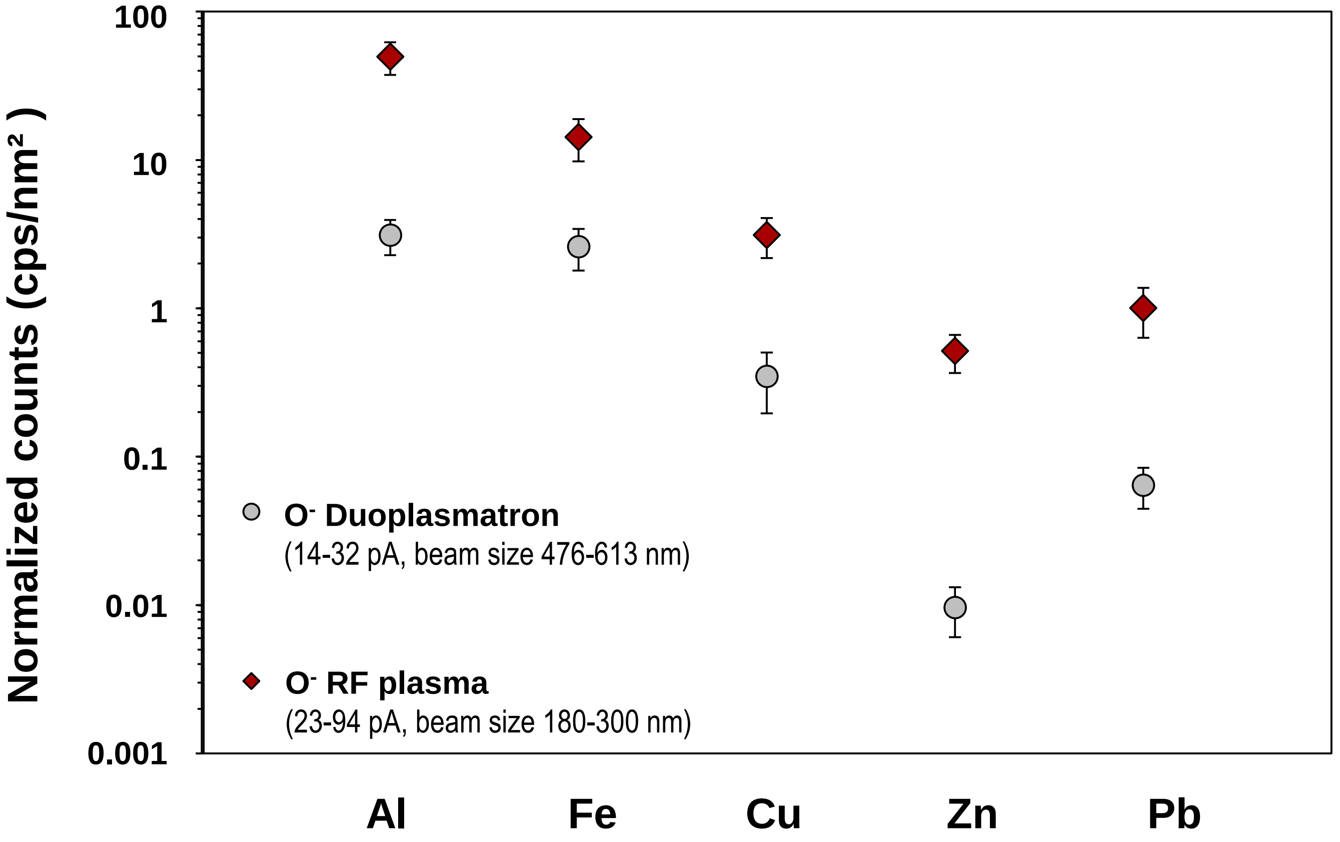


Current density at the sample **16 times** higher with RF plasma source

Achievable lateral resolution improved by a factor of **3**

Comparison of normalized counts for selected elements using O^- duoplasmatron and O^- RF sources

Count rate normalized to acquisition time, probe size, and isotope abundance



30x30 µm
256x256 pixel

reference materials

Increased secondary ion yield ⇒ increased apparent element sensitivity (by factor 5 to 45)

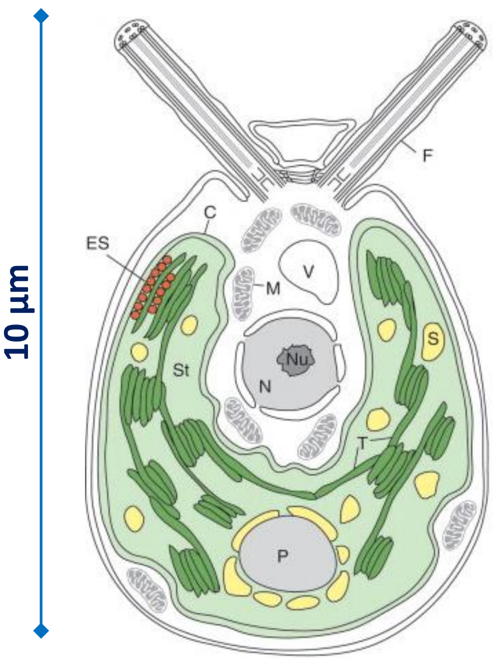
Bioimaging with NanoSIMS

Use of the RF plasma oxygen primary ion source for the localization of major (**Na, Ca, P**) and trace (**Fe, Cu,**) elements in plant (algae) cells

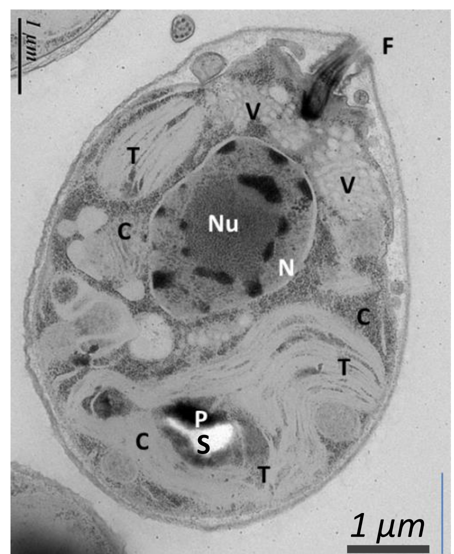
Application to a model organism

Model system: *Chlamydomonas reinhardtii*

- single celled green micro algae
- commonly found in soil and fresh water
- exists in different strains
- model organism to study cell response to metal stress



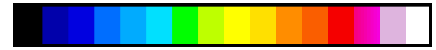
F : **Flagella**
V : **Vacuoles**
N : **Nucleus**
Nu : **Nucleolus**
C : **Chloroplast**
T : **Thylakoid**
P : **Pyrenoid**
S : **Starch**



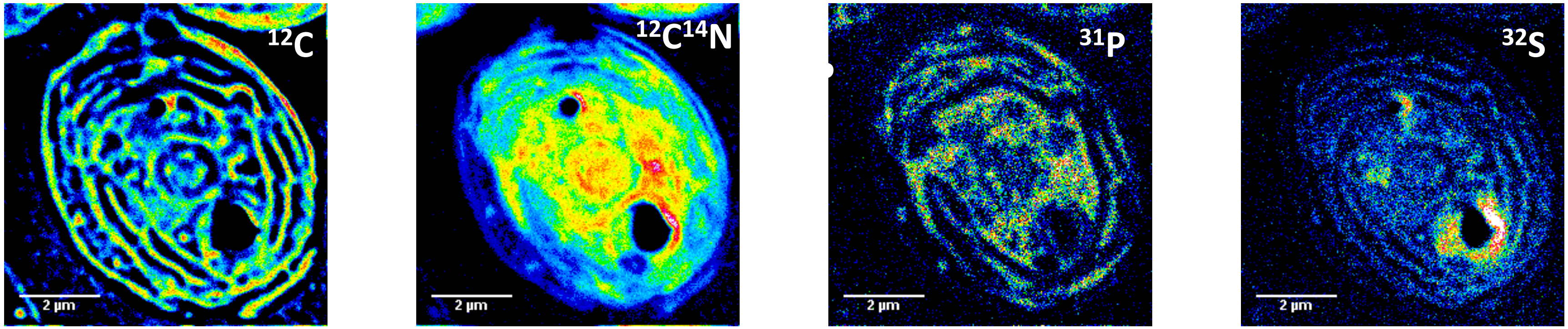
TEM analysis
(70 nm thin section)
resolution down to 1 nm

Cs^+ source

256pix 8µm 1 pA 10ms/pix



[min-max]

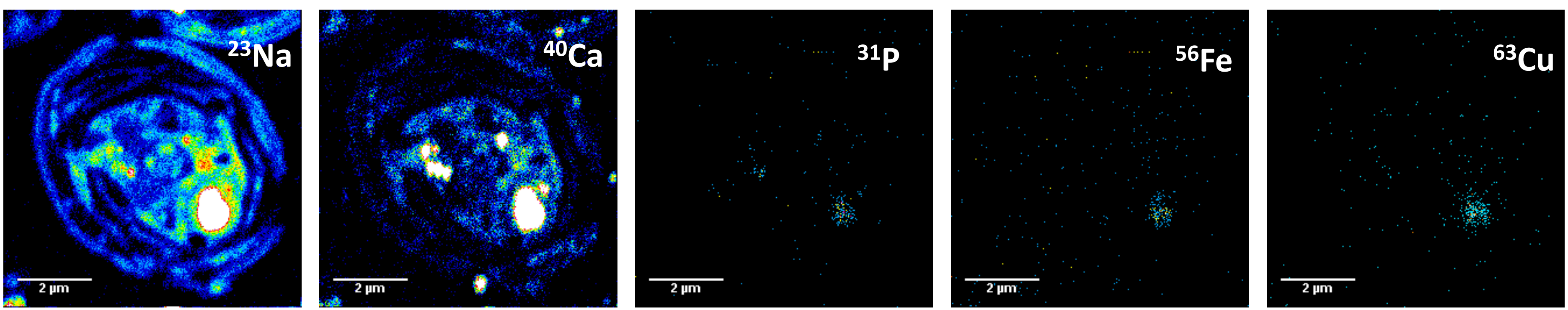


[800-1500] [75-500] [0-14] [14-60]

O^- RF plasma source

256pix FOV 8µm 1,4pA 10ms/pix

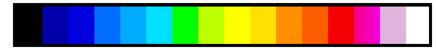
[min-max]



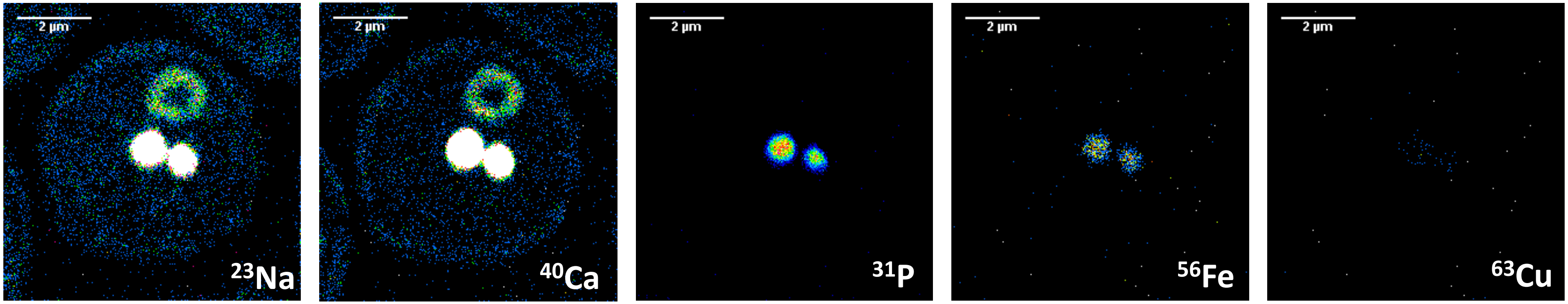
[0-721] [0-331] [0-4] [0-4] [0-4]

O^- Duoplasmatron source

256pix FOV 8µm 1,5pA 8ms/pix

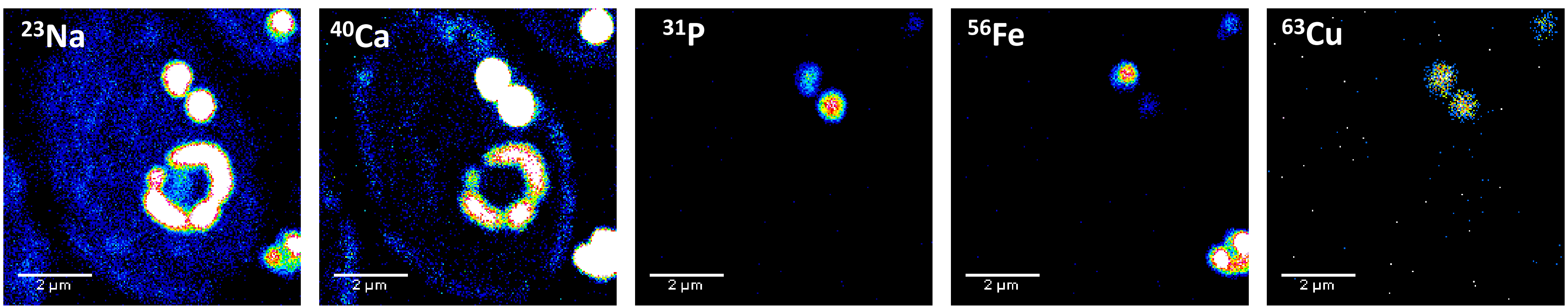


[min-max]



[0-95] **[0-144]** **[0-38]** **[0-6]** **[0-5]**

256pix FOV 8µm 1,5pA 15ms/pix



[0-229] **[0-1771]** **[0-68]** **[0-118]** **[0-7]**

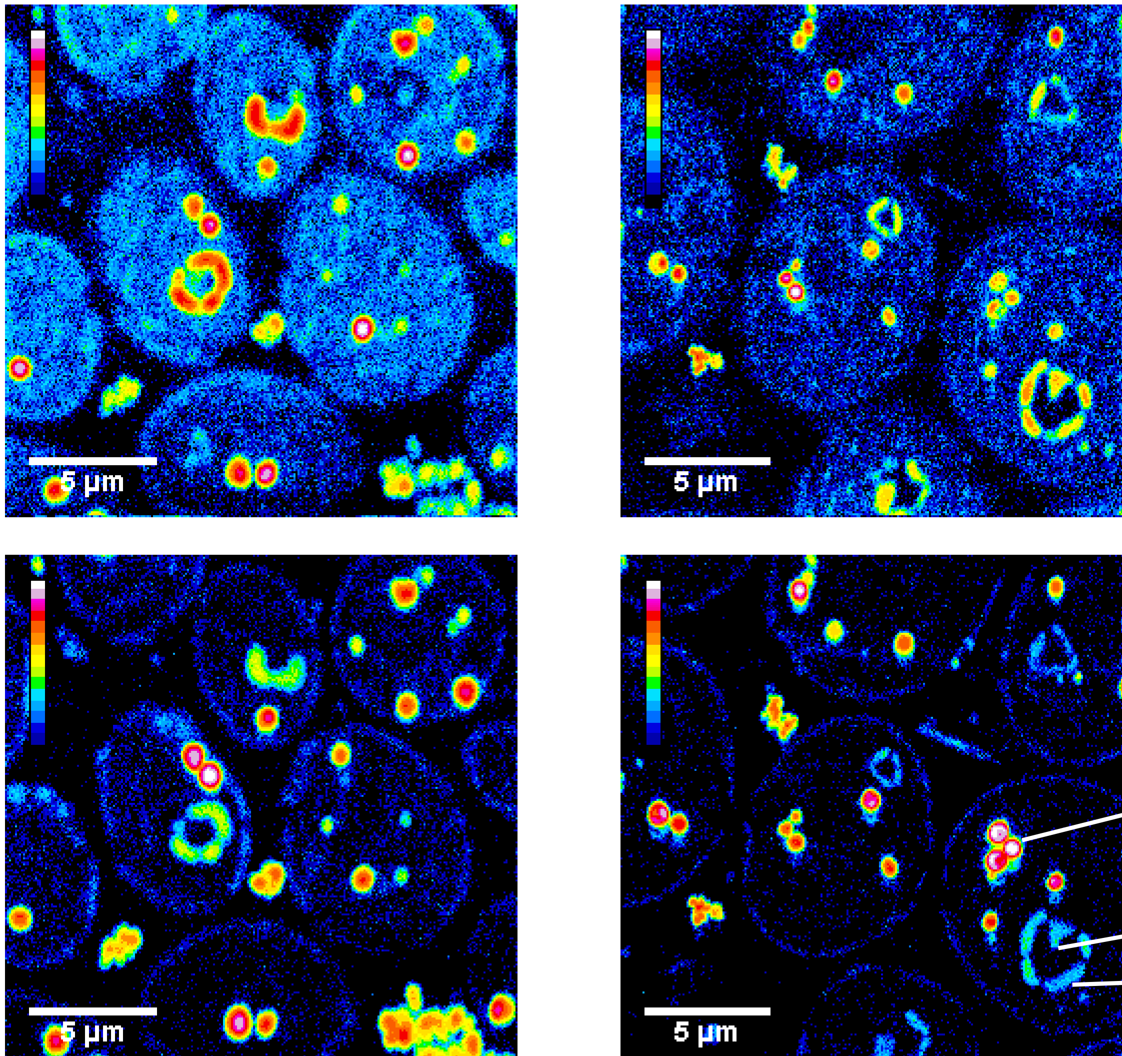
NanoSIMS analysis of *Chlamydomonas reinhardtii* cells

Comparison conventional Duoplasmatron O$^-$ ion source and new RF plasma O$^-$ ion source

Duoplasmatron O- ion source | **New RF plasma O$^-$ ion source**

^{23}Na

^{40}Ca



acidocalcisomes

pyrenoid with starch plates

300 nm
thin sections

relative intensity: Max – Min

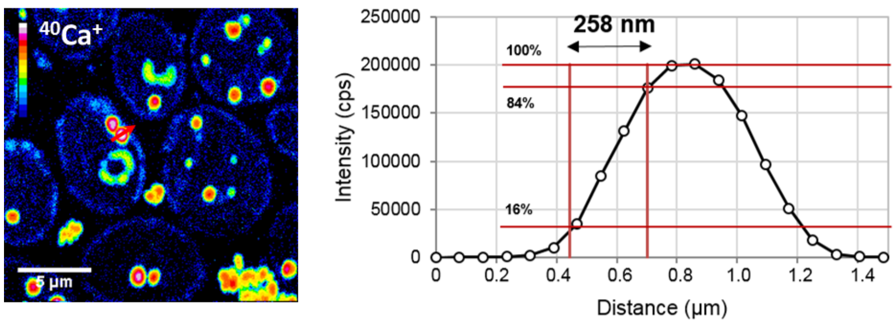
20 x 20 µm
11 min (Duo)
5.5 min (RF)
256x256 pixel
1 plane

Lateral resolution in biological cell imaging (*C. reinhardtii*)

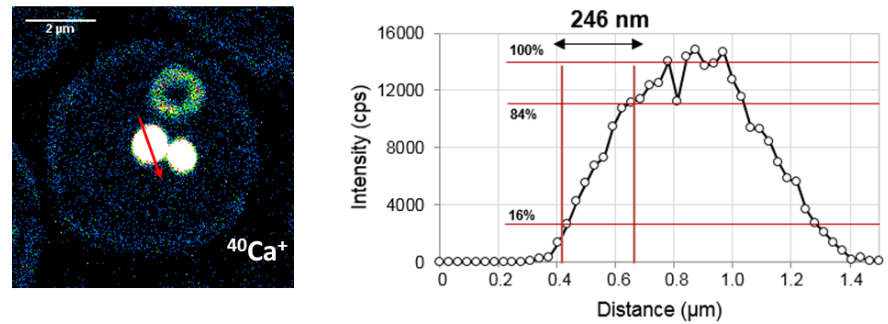
Line scans on Ca containing vacuoles/acidocalcisomes

O^- Duoplasmatron

2.5pA ; FOV 20µm ; 256pix ; 10ms/pix

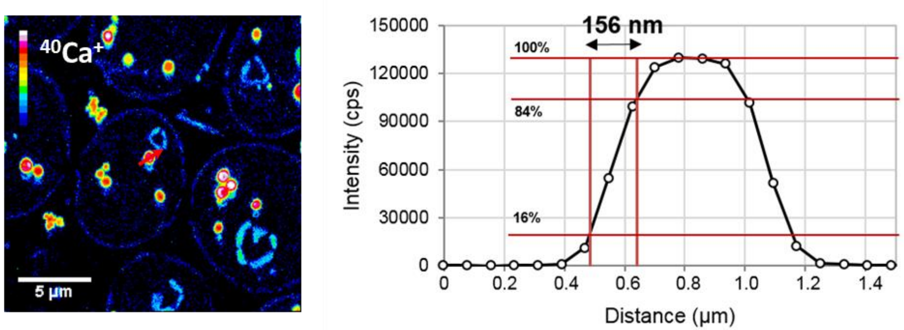


1.5pA ; FOV 8µm ; 256pix ; 8ms/pix

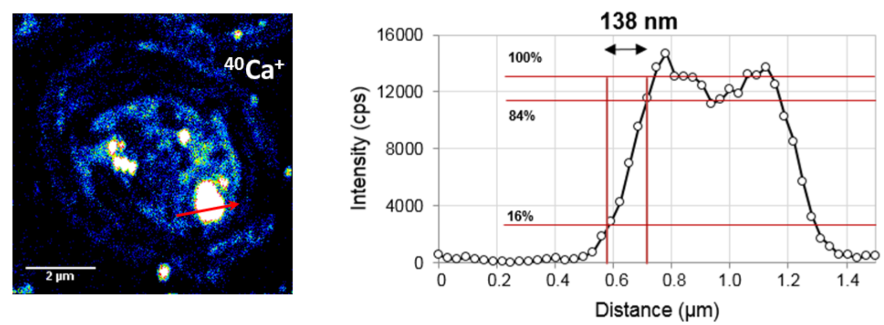


O^- RF plasma

86pA ; FOV 20µm ; 256pix ; 5ms/pix

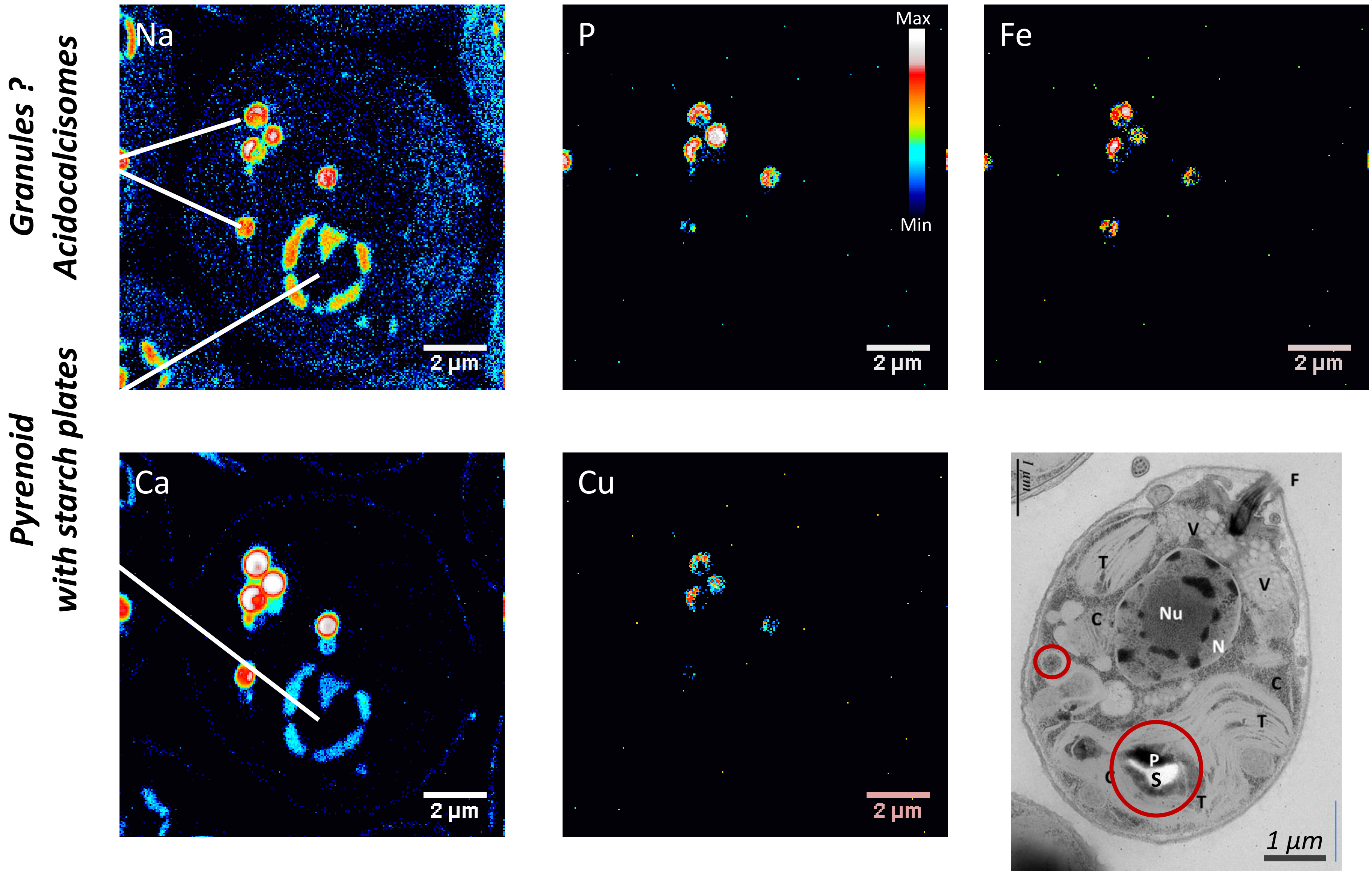


1.4pA ; FOV 8µm ; 256pix ; 10ms/pix



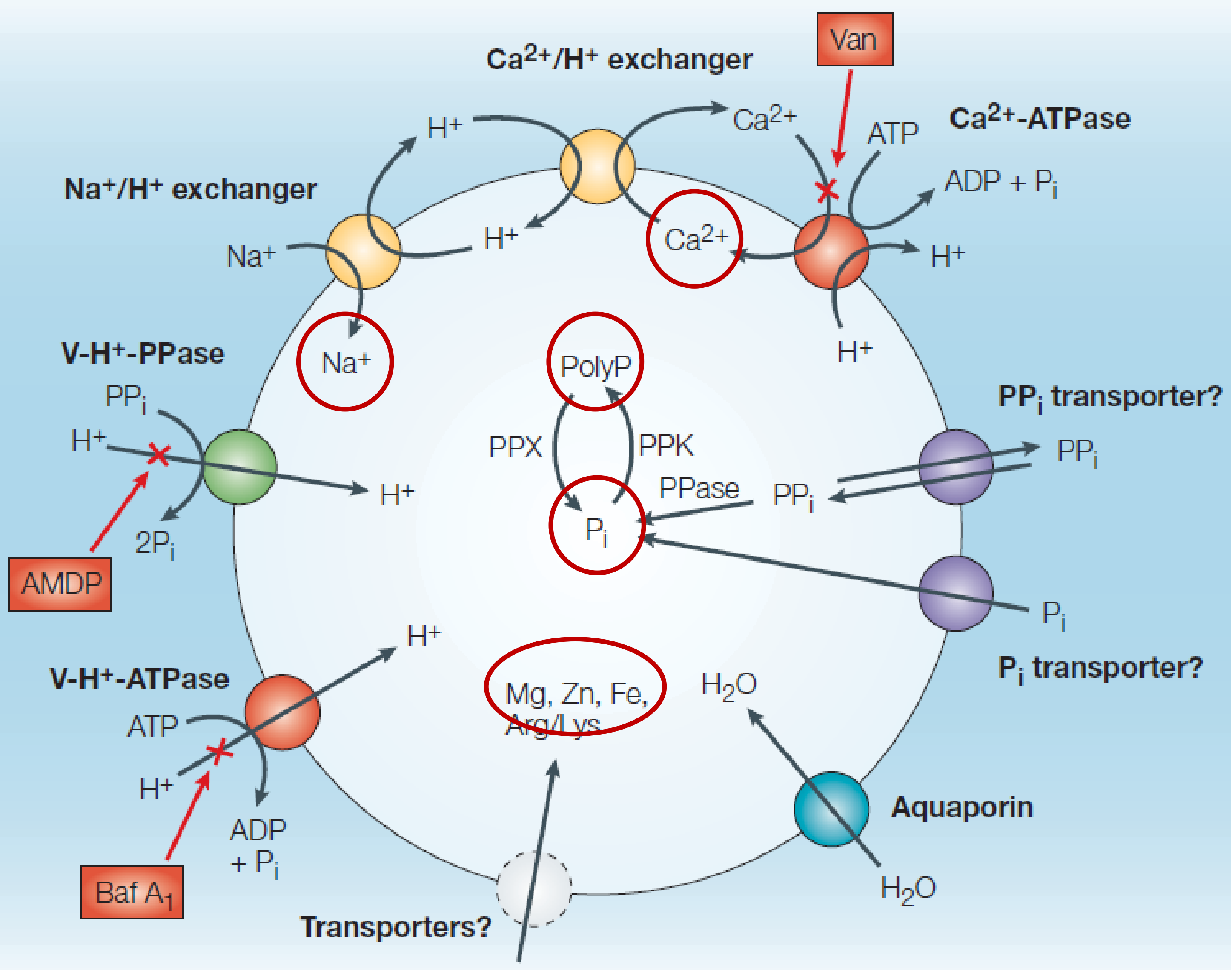
[min-max]

Subcellular element imaging by NanoSIMS (RF plasma O⁻ ion source)



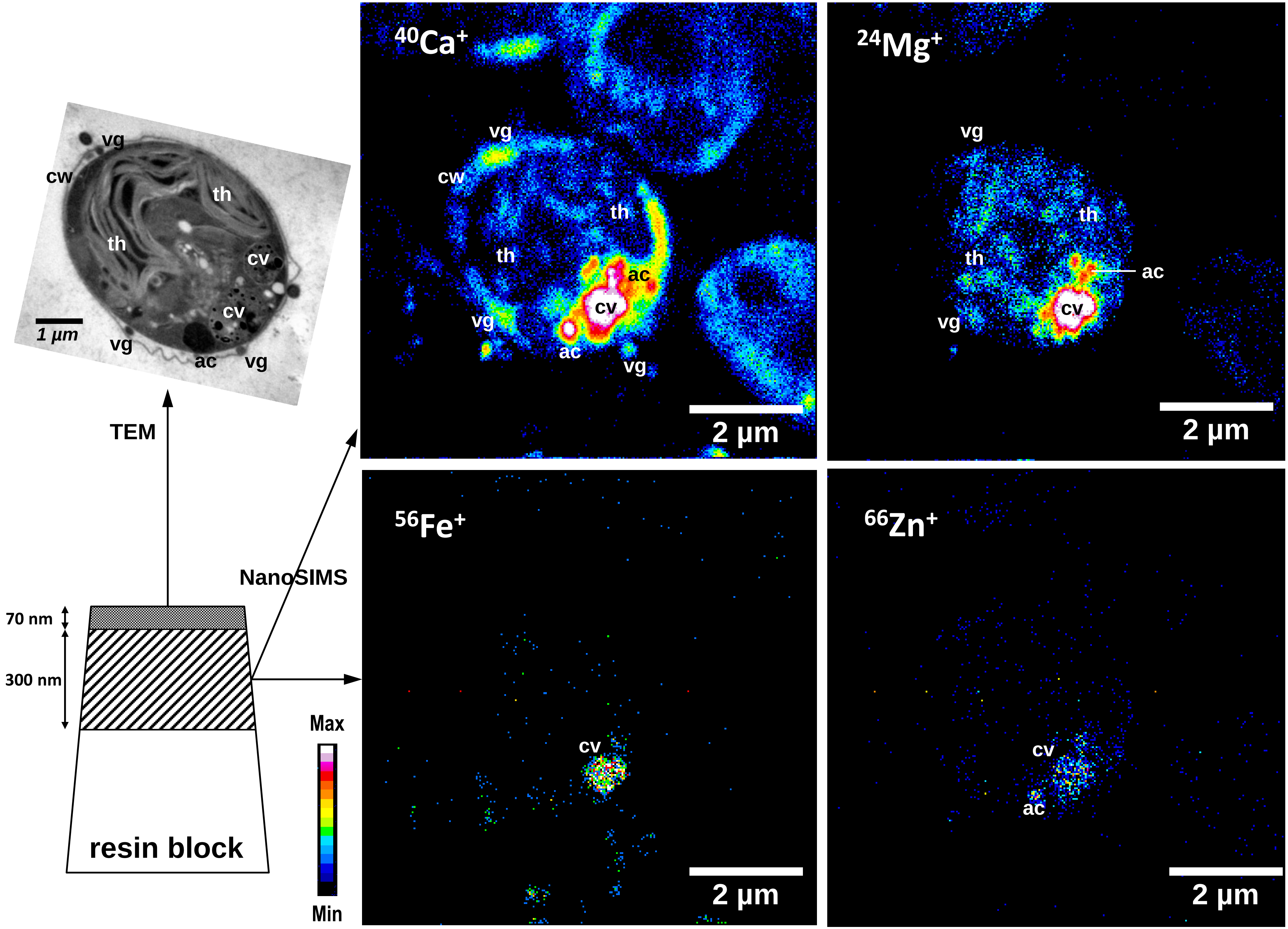
Single cell imaging: 12 x 12 µm, 22 min, 512x512 pixel, 5 ms/pixel 2 planes

Scheme of a Acidocalcisome



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

Correlative imaging TEM - NanoSIMS



RF plasma O⁻ source: 10 × 10 µm FOV; 256 × 256 pix; dwell time 25 ms/pix; 27 min.

Conclusions: advantages of the new RF plasma 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 % variation of primary current over 14h
- High resolution images of **trace elements in biological cell** opens new application fields

Acknowledgements

University of Pau/CNRS-IPREM

Florent Penen	PhD student
Marie-Pierre Isaure	Lecturer
Anne-Laure Bulteau	CNRS researcher (now ENS Lyon)

University Potsdam (Germany)

Tanja Schwerdtle	(neurotoxicology)
Julia Bornhorst	(neurotoxicology

- French ANR-EQUIPEX program (Equipment of Excellence)
  Project: **ANR-11-EQPX-0027 – Mass Spectrometry Center MARSS**
- CAMECA
- French Ministry of Research (PhD fellowship)
- Campus France – DAAD


Thank you for
your attention !