PATROLS
Advanced Tools for NanoSafety Testing

Methodologies for characterizing complex in vitro/in vivo/ecotox systems

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SIAM Synthesis Irradiation & Analysis of Materials

SIAM Platform - unique facilities

UNIVERSITÉ DE NAMUR

ANALYSIS

- **Tandetron Linear Accelerator (ALTAÏS) – Characterization**

  IBA non-destructive and quantitative elemental depth (10nm to 1µm) profiles (H quantification).
- **XPS and ToF-SIMS**

  Chemical and molecular composition at surfaces

  Chemical mapping (2D) and profiling (3D)

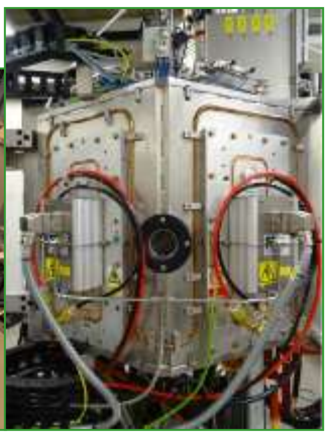
  Polymers depth profiling

SYNTHESIS & IRRADIATION

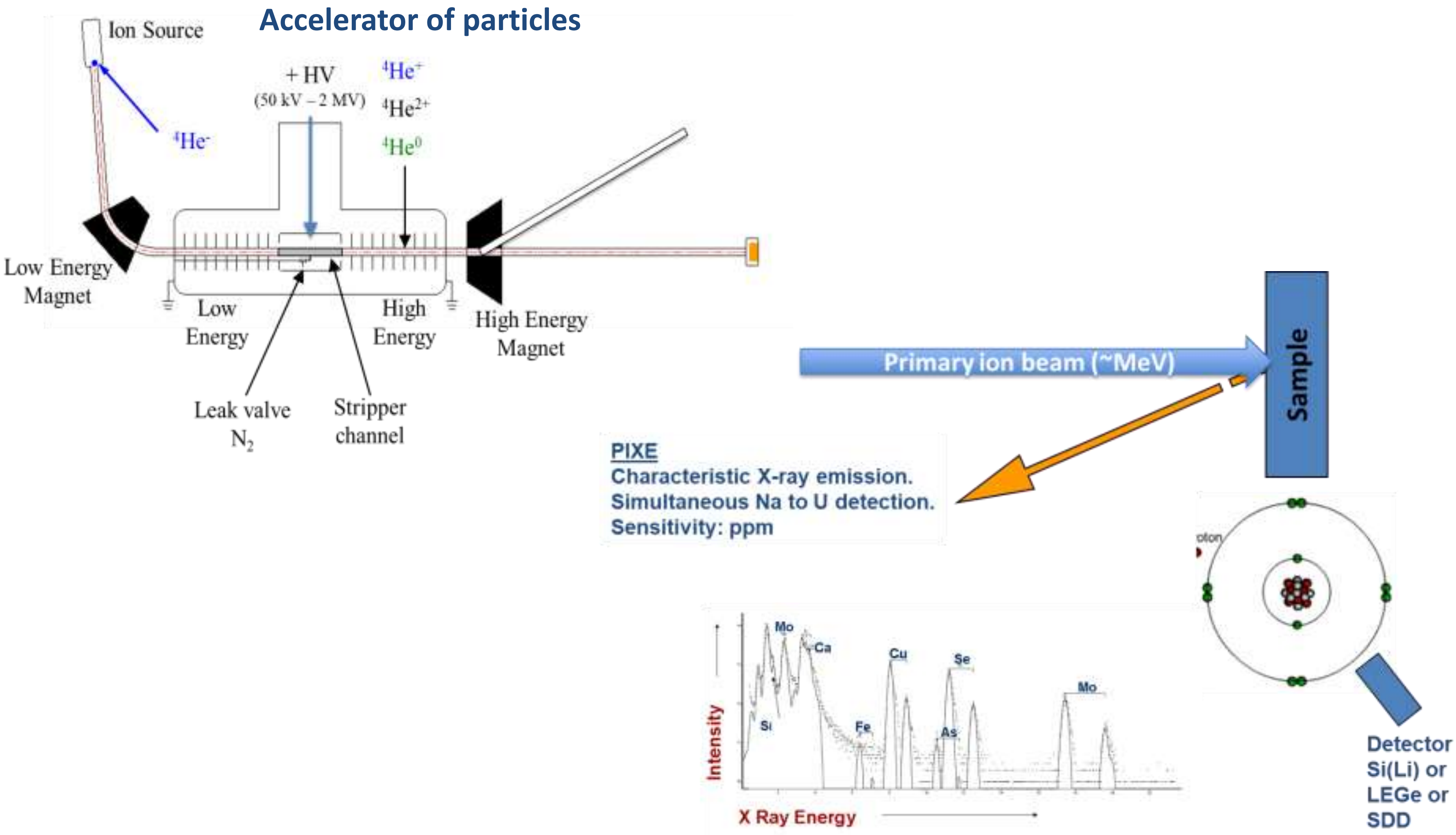
Tandetron Linear Accelerator (ALTAÏS) - Irradiation

Radiobiology station (cells irradiation)
Implantation station
Vacuum deposition chambers

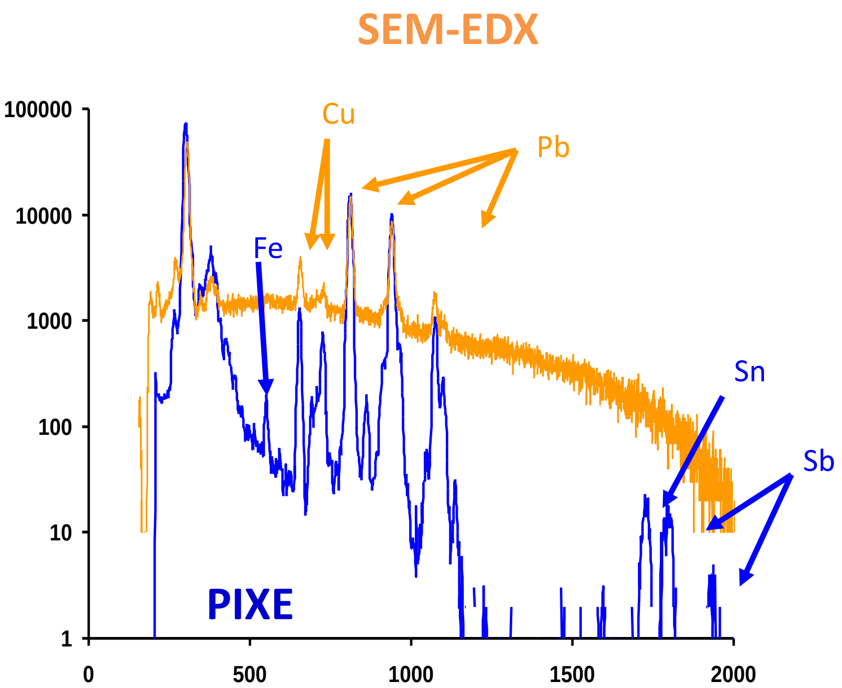
Plasma sputtering
Plasma functionalization



Ion Beam Analysis - Particle-Induced X-Ray Emission (PIXE)



Benefits of PIXE



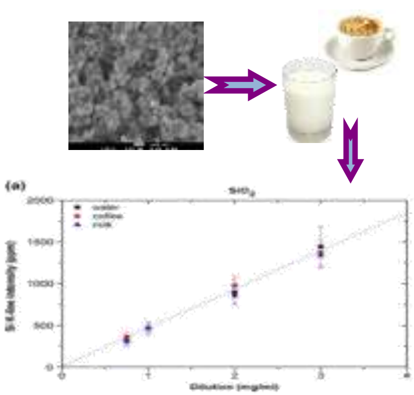
- Detection **from Na to U.**
- Sensitivity at **wt.ppm level** for most elements.
- **PIXE** is much more sensitive to trace elements than the **electron micro-probe**, **no Bremsstrahlung**
- Analysis can be carried out **in air or in vacuum.**
- Generally **no need of sample preparation.**
- **Absolute quantification** (need to compare to standards).
- The **accuracy** of the technique is **5-10%**.

PIXE at UNamur since 1970.

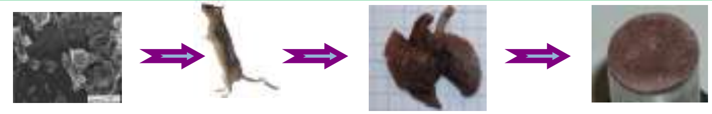
PIXE / µ-PIXE: Applications to NanoSafety

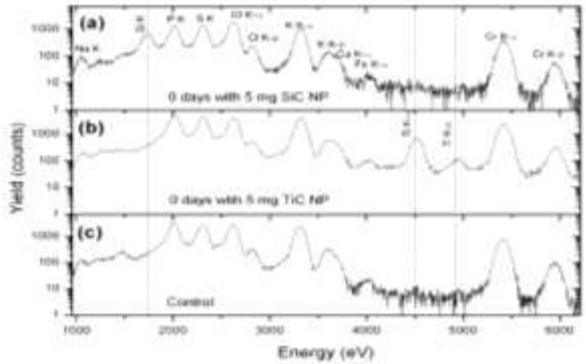
Characterisation of NMs

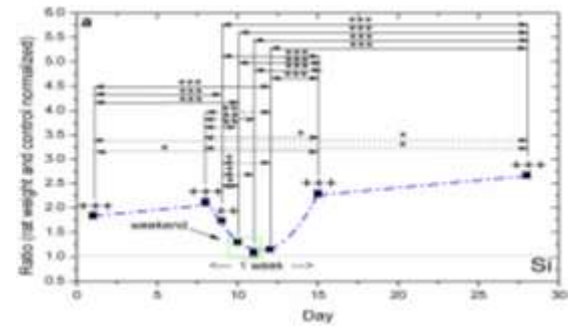
Quantification of NMs in complex media
(i.e. Quantification of SiO_2 NPs in water, coffee and milk)



Biodistribution and biopersistence (*in vivo*) of NMs
(Biopersistance of NPs in rat organs)

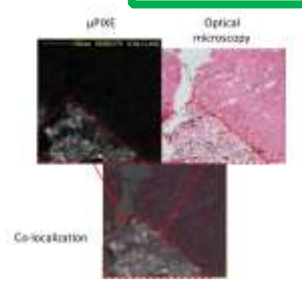


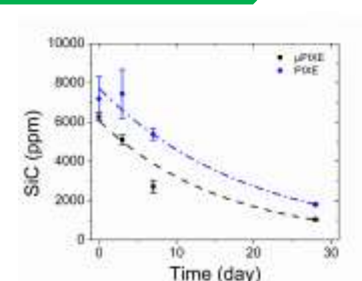




Lozano *et al*,
"Effects of SiC nanoparticles orally administered in a rat model: Biodistribution, toxicity and elemental composition changes in feces and organs" **Toxicology and Applied Pharmacology 264 (2012) 232–245**

2D mapping - µPIXE
(Localization of MNs)





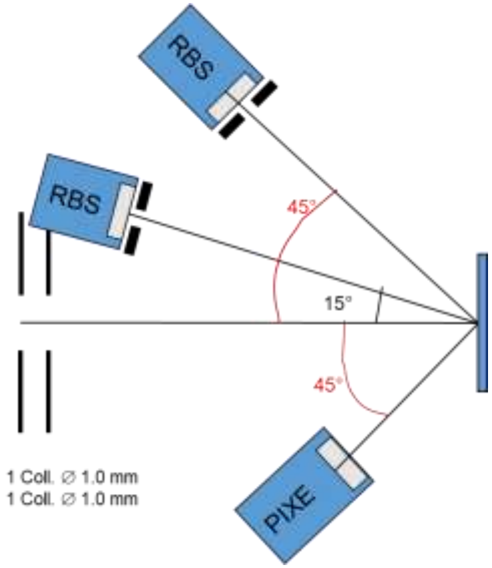
O. Lozano, J.L. Colaux *et al*.,
"Fast, asymmetric and non-homogeneous clearance of SiC nano-aerosol after 5 day exposure using ion beam analysis" **Nanomedicine** (2017)

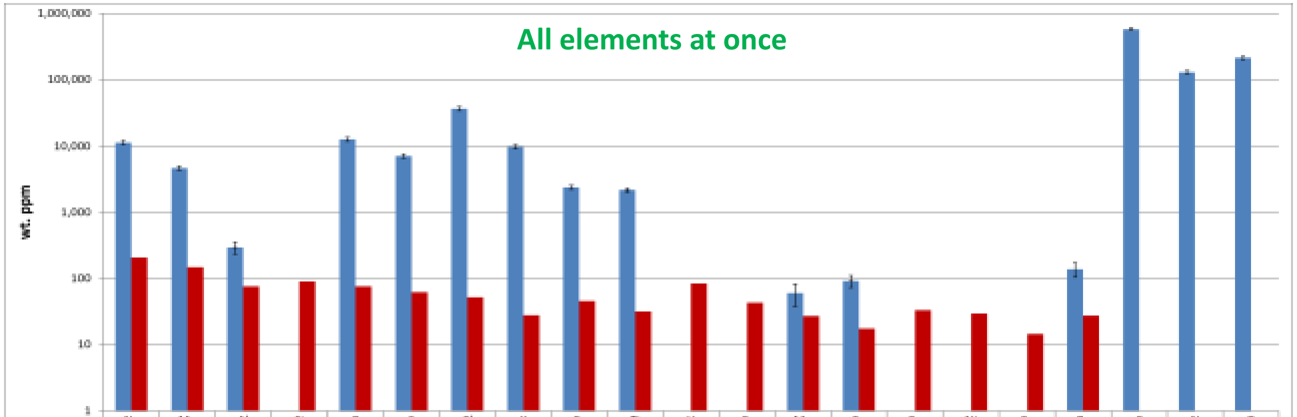
***in vitro* safety assessment**
(Different cells lines exposed to MNs)

Zebrafish eggs samples (LU)

Exposure to TiO_2 NPs (NM-105) in ecotox media (M7, egg water)

Experimental setup (2,5 MeV)

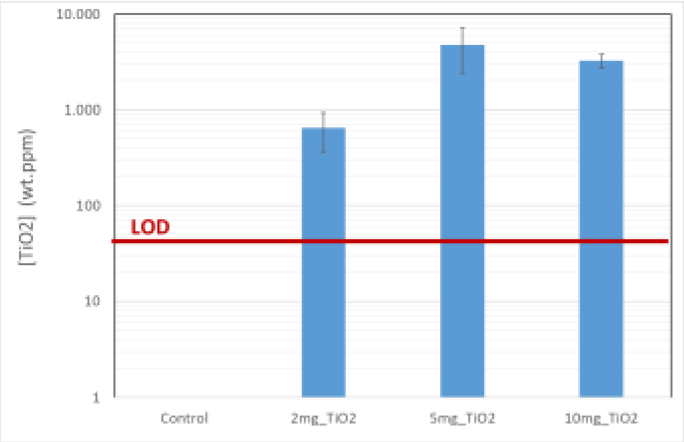




	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	C	N	O
Conc.	11,277	4,626	292	0	12,599	7,092	35,950	9,731	2,375	2,154	0	0	60	91	0	0	0	136	573,694	128,076	211,847
LOD	204	147	75	90	75	61	51	28	45	31	84	43	27	17	33	29	14	27			

Solid samples!

=> "pellets"



LOD = 40 wt.ppm

=> Must be improved for ecotox samples

Potential for simulation (data low uncertainty).

Translocation experiments (apical, basal, membrane, cells). Samples provided by AMI.

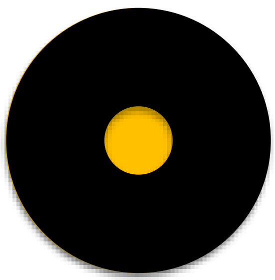
Liquid sample and configuration settings

Sample holder

Side view

Carbon tape
Thin gold layer (~15 nm)
Vitreous carbon

Top view

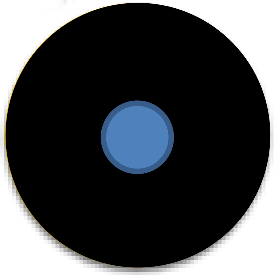


The hole in carbon tape is 3 mm in diameter

(1)

Sampling specific volume (5-10 µL)

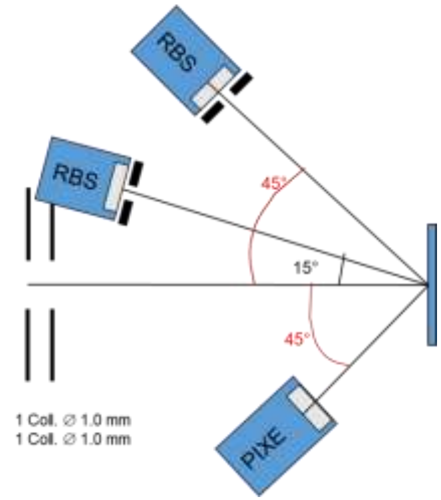
(2)



Deposition on controlled area

(3) Drying (oven 60 °C)

(4) IBA analysis



NM-220 ($BaSO_4$), NM-105 (TiO_2), NM-212 (CeO_2), NM-110 (ZnO_2)

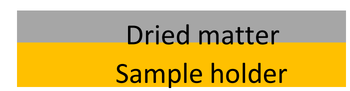
Liquid sample results (AMI)

	sample	[Ba] wt.ppm	Unc. Wt.ppm	LOD wt.ppm
UNamur	2mg_BaSO4	612,219	39,251	1,227
UNamur	0.1mg_BaSO4	11,301	1,639	2,861
UNamur	0.01mg_BaSO4	296,200	94,441	2,659
UNamur	0.001mg_BaSO4			1,664
PATROLS	2mg_BaSO4	561,051	36,446	2,042
PATROLS	0.1mg_BaSO4	29,721	3,118	2,541
PATROLS	0.01mg_BaSO4	4,672	2,317	2,733
PATROLS	0.001mg_BaSO4			2,691

	sample	[BaSO4] mg/ml	Unc. mg/ml	LOD mg/ml
UNamur	2mg	1.708	0.1095	0.003
UNamur	0.1mg	0.031	0.0045	0.004
UNamur	0.01mg	0.277	0.0882	0.001
UNamur	0.001mg			0.002
PATROLS	2mg	1.266	0.0822	0.005
PATROLS	0.1mg	0.169	0.0178	0.014
PATROLS	0.01mg	0.021	0.0102	0.012
PATROLS	0.001mg			0.012

Uncertainty values are established from a bottom-up approach (uncertainty budget).

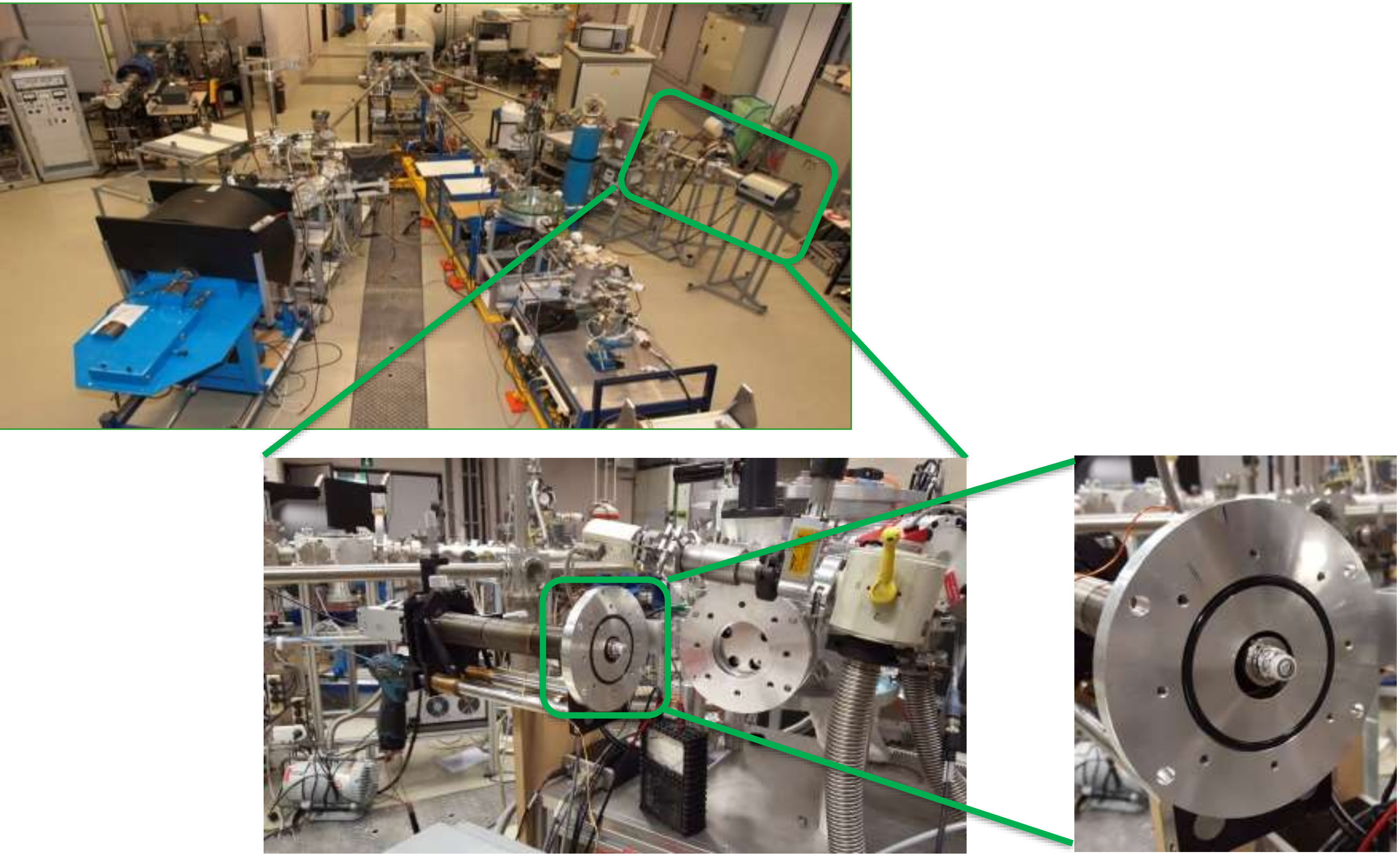
Ideally we should get this:



However:

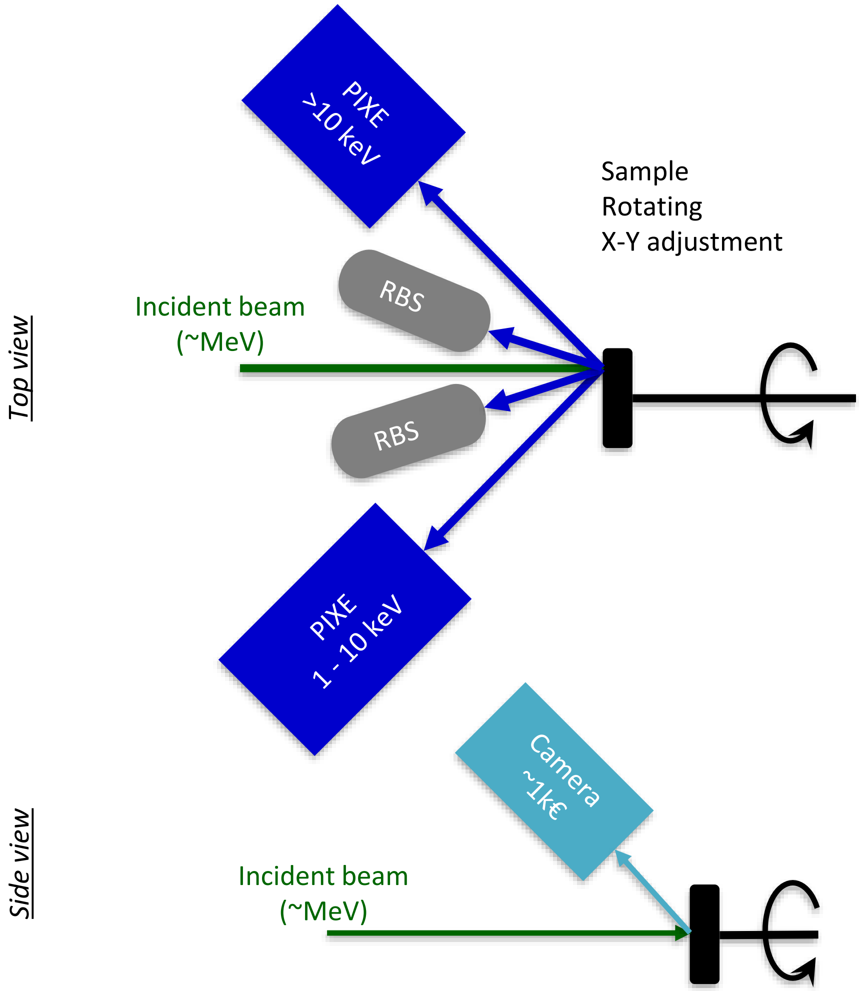
Moreover, LOD still too high!

Measurement of liquid sample by IBA



Measurement of liquid sample by IBA

Improving LOD
(set-up under development)

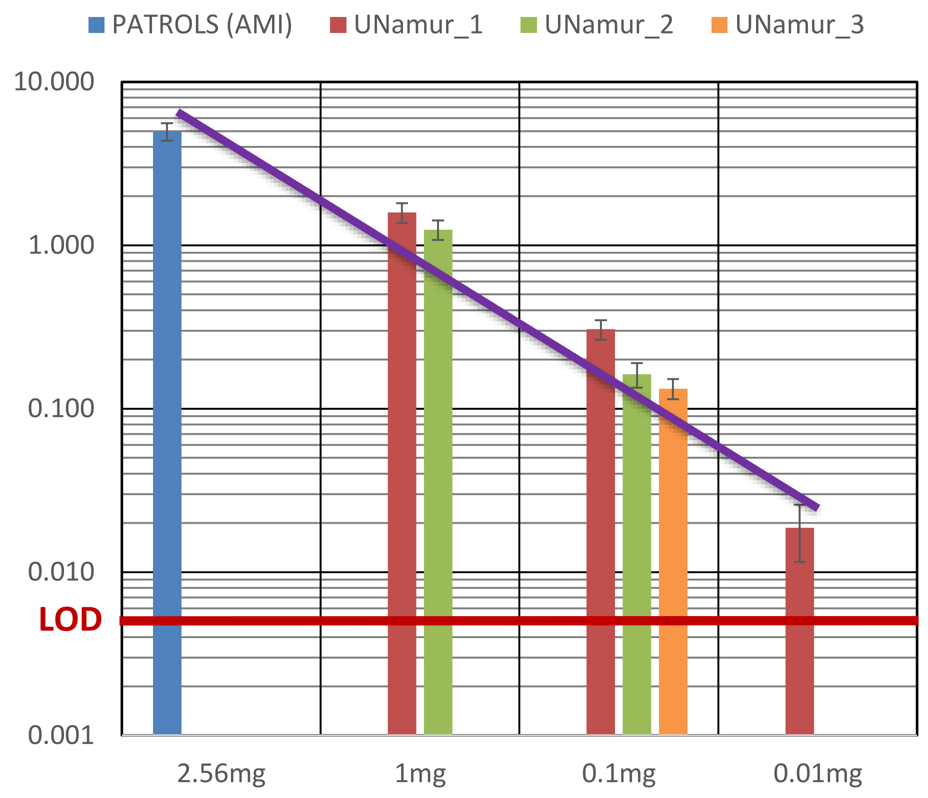


Improving sample uniformity

Cells for liquid sample (50 µL)

Some results

NM-105 (TiO_2)



Conclusions so far

- Liquid samples measurements improved: minimal manipulation and small volumes (~50 µL).
- Improvements to the geometry are still possible. LOD reduced by a factor 2.
- Actual LOD is about 0,05 mg/mL, sample concentration is required for lower concentrations.
- Data from the NMs and also the matrix in a same run.
- Cross check on the NMs dispersion protocol (exposure concentrations).
- Low uncertainty values and potentially valuable data for simulation purposes.
- High potentiality for analyses of complex samples (in vitro/in vivo/ecotox, …)

Perspectives

- A sample stability's study will be initiated soon.
- Liquid samples from partners are planned in the following weeks and months.
- Improve sample preparation for solid samples

Thank you for your attention

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 760813.

Values given are indicative or typical "best" values.

Different applications may have widely differing performances.

Possible primary beam energies and types are indicated. A wide variety may be used.

	SIMS	XTEM	SAM	GD-OES	XPS	LA - ICP-MS	IBA
Primary beam	keV ions	~100 keV electrons	~100 keV electrons	plasma	X-rays	Pulsed laser	~3 MeV light ions ~30 MeV heavy ions
Detected signal	Sputtered ions	Primary electrons in phase contrast	Auger electrons	visible photons	Photo-electrons	Evapor-ated ions	X-rays; Nuclear reaction products: scattered primaries, target recoils and γ-rays
Destructive	Yes	Yes	Yes	Yes	Yes	Yes	No
Depth resolution	2 nm	0.1 nm	2 nm	20 nm	2 nm	10 nm	2 nm
Information depth	500 nm	100 nm	500 nm	50 µm	500 nm	--	15 µm
Lateral resolution	50 nm	0.1 nm	2 nm	1 mm	3 µm	10 mm	500 nm
Elemental Imaging	Yes	EELS, EDX	Yes	No	Yes	No	Yes
Ambient analysis	No	No	No	No	No	Yes	Yes
Sample preparation	No	Yes	UHV	No	UHV	No	No
Quantitative	?	No	Yes	Yes	Yes	Yes	Yes
Standards needed	Yes	--	Yes	Yes	Yes	Yes	No
Elemental sensitivity	10^{-8}	10^{-1}	10^{-3}	10^{-6}	10^{-3}	10^{-9}	10^{-6}
Accuracy	--	--	10%	10%	5%	5%	1%
Traceability	--	--	--	--	Yes	Yes	primary

C. Jeynes & J.L. Colaux, *"Thin film depth profiling by ion beam analysis"*, **Analyst 141 (2016),** 5944-5985.

Interactive Map of Accelerators in the World

The map view is optimized for Internet Explorer. If the map is not loading in Chrome/Safari then please kindly refresh the page

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Accelerator Knowledge Portal

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