# Nanoscopic Quantitative X-ray Fluorescence Imaging of Cells with a High Energy X-ray Cryo Nano-probe.

Alexandra Pacureanu[1], Yang Yang[1], Murielle Salomé[1], Julio Cesar da Silva[1], François Villard[1], Lionel André[1], Peter Van Der Linden[1], Peter Cloetens[1], Sylvain Bohic[1,2,*]

[1] European Synchrotron Radiation Facility, 71 avenue des Martyrs 38000 Grenoble, France.
[2] Inserm, UA7, Synchrotron radiation for Biomedicine, STROBE, Université Grenoble Alpes, Grenoble, France.
* Corresponding author, bohic@esrf.fr

Several essential metal ions participate in the control of numerous metabolic and signaling pathways, but their rich coordination chemistry and redox properties confer them a propensity to randomly coordinate and catalytically react inside the cell with protein sites other than those tailored for that purpose. A number of sophisticated networks of trafficking pathways are available to tightly regulate their uptake, intracellular transport and compartmentalization, and to avoid their toxic side effects. Cutting-edge technique providing quantitative imaging for detailed study of elemental homeostasis or the intracellular distribution of metal-based drugs at biologically relevant concentration in a label-free fashion is highly desirable. The synchrotron X-ray fluorescence (XRF) nanoprobe as developed today provide the required sensitivity and spatial resolution to elucidating the 2D and 3D distribution, concentration of elements particularly metals inside entire cells at the organelle level. The new state-of-the-art Nano-Imaging beamline ID16A-NI at ESRF offers unique capabilities for X-ray imaging at nanometer scale delivering an extremely bright, nanofocused beam ($> 5\times10^{11}$ph/s at $\Delta\lambda/\lambda\sim10^{-2}$) at high energies (~30nm at 17kev) [1]. It is particularly well suited for the investigation of biological samples at high spatial resolution, e.g. combined hard X-ray phase imaging and XRF detection and quantification of trace elements [2]. A critical issue is to best preserve the structural and chemical integrity of the cells. As it has been demonstrated in electron microscopy [3,4] or recently for synchrotron XRF [5,6], a cryogenic workflow including cryo-immobilization of the cell and cryotransfer to a cryo-scanning stage allow an optimal elemental preservation at subcellular level as close as possible to their native state. Therefore, the implementation of a cryo-environment becomes critical and additionally provide an effort to reduce structural radiation damage. The new ID16A beamline implements a 3D cryogenic framework from sample preparation to nanoprobe analysis. An integrated cryostat and cryogenic transfer system operating under high vacuum have been installed that allows nanochemical imaging of frozen-hydrated samples at 120 K. In this work, we will illustrate the capabilities of this techniques to provide quantitative nanoscopic cryo-XRF of cell as diverse as cancer cells exposed to organometallic drugs[6], neurons (in press) or cancer cells exposed to metal-based nanoparticles and we will try to underline the importance of correlative sub-cellular imaging for better understanding of the role of metals in cells.

References:
[1] J. C. Da Silva *et al*, Optica **4** (2017) 492
[2] Bohic S. *et al.*, Journal of Structural Biology, **177** (2012) 248-258
[3] Saubermann, A.J. and Heyman, J. Microsc. **146** (1987) 169-182
[4] Wroblewski, J., *et al*, Histochemistry (1983) **77** (1983) 447-463
[5] Perrin L. *et al*, JAAS **30** (2015) 2525-2532
[6] Fus F. *et al*, Angwendte Chemie. (2019) doi: 10.1002/anie.201812336