ESRF	**High spatial resolution mapping of metals related to Parkinson's disease etiology**	**Experiment number**: MD80
Beamline: ID19	**Date of experiment**: from: 21/04/2004 to: 27/04/2004	**Date of report**: 11/03/2005
Shifts: 15	**Local contact(s)**: Peter CLOETENS	*Received at ESRF:*

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The aim of this experiment was to perform chemical element mapping on dopaminergic neurons, PC12 cultured cells, at the sub-cellular level, in order to identify intracellular sites of metal accumulation in relation to Parkinson's Disease (PD) etiology. PD is a neurodegenerative disorder that leads to the progressive loss of dopaminergic neurons in the substancia nigra. This phenomenon is still unexplained, even though some metals, and especially redox metals such as Mn and Fe are suspected to play a major role in the etiology of PD. In this study PC12 cells were continuously exposed to L-dihydroxyphenylalanine in order to produce neuromelanin, a pigment typically found in dopaminergic neurons of the substancia nigra. Neuromelanin is suspected to bind Fe and toxic metals that could promote neurodegeneration.

In this experiment, an original setup for high spatial resolution X-ray fluorescence microanalysis based on a Kirkpatrick-Baez lens and a piezo sample stage was implemented on ID19 beamline (Figure 1), thus leading to the obtention of a photon beam at the same time highly spatially resolved (100 nm beam size) and with a high flux of photons (10^{11} ph/s). The characteristics of the beam fullfilled the requirements for mapping of biological trace elements (in the µg/g range) at a sub-micrometer scale (size of most intracellular organites). The energy of the incoming X-ray beam was set at 14.8 keV allowing to map all trace elements distribution in cells.

The first part of the allocated beamtime was dedicated to the optimization of the fluorescence set-up in terms of sample geometry, scanning step size, beam stability. An optimum was found with a very compact geometry and a 200 nm step scan (horizontally and vertically) that allowed to maintain sample integrity and to record chemical maps with a sufficient spatial resolution to achieve intra-cellular organites mapping. The beam was found to be stable over a period of several hours as needed for the mapping of a single cell: a typical required time for the mapping of a synapse was 30 min, 45 min for axons and several hours were necessary for cell bodies.

The second part of the experiment was used to map all cellular compartments (nucleus, cytoplasm, axons and synapses) of PC12 dopaminergic neurons. An example of K and Fe iron distributions obtained on the cell body with a spatial resolution of 200 nm is presented in figure 2. The figure shows an homogeneous K distribution, in agreement with the known distribution of K in cells and an heterogeneous Fe distribution with a marked difference between cell nucleus and cytoplasm. The granular distribution of Fe could be related to cellular sub-structures such as neuromelanin granules. The Fe profile distribution (figure 3) as retrieved from the region zoomed in figure 2, confirms that Fe is localized in structures of a few hundreds of nm scale in the cytosol, similar in size and intracellular localisation to neuromelanin granules. The chemical maps obtained during the experiment are the best ever achieved in terms of spatial resolution and bring in-situ evidence for the relation existing between the chemical distribution and sub-cellular structures.

Our results strongly suggest that Fe is complexed to neuromelanin granules in catecholamine producing cells. In the future, this unique X-ray fluoresence high resolution set-up would be of paramount interest to prove experimentally the existence of iron-dopamine intracellular complexes, not only in neuron cell bodies, but also in neurosecretory vesicles at the synapse, as suggested by results obtained on synapse chemical imaging (data not shown).

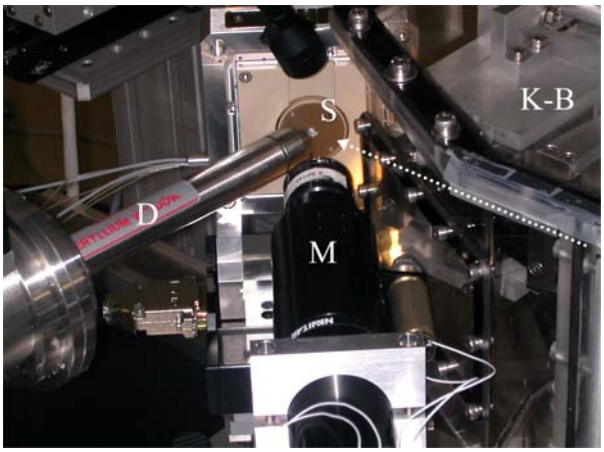

Figure 1. The scanning X-ray fluorescence set-up implemented on ID 19 beam line showing at the exit of the Kirkpatrick-Baez optics (K-B) the positions of the sample (S), the X-ray fluorescence detector (D) and the optical microscope (M) relative to the photon beam (dotted arrow).

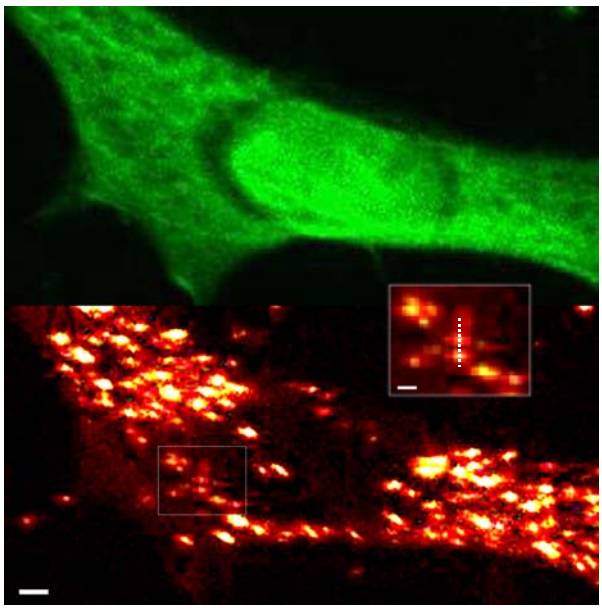

Fig 2. Chemical maps of K (green) and Fe (red) of a single PC12 neuron. Note the homogeneous distribution of K, the nucleus and cytosol are clearly observed. Iron is found in sub-micrometer structures located in the cell body, but not in the nucleus, suggesting a complexation of Fe in neuromelanin granules.

Scale bars: 2.5 µm (main frame) and 1 µm (zoom).

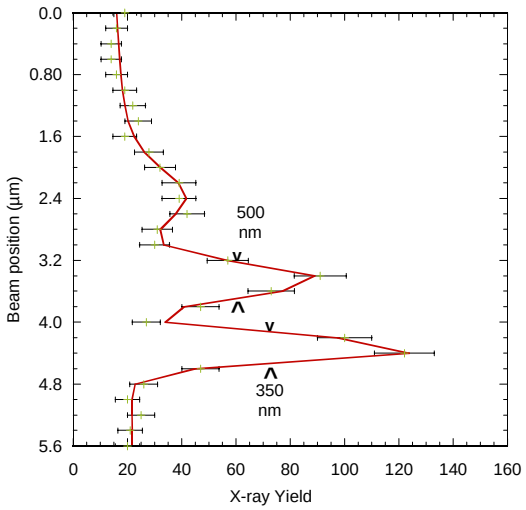

Fig 3. Iron profile at the position of the dotted line in zoom from figure 2. Structures as small as 350 nm could be resolved. This size is typical of neuromelanin granules in dopaminergic neurons.