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Multimodal Correlative Microscopy: combination of ion beam micro-analysis with complementary microscopy techniques for the study of nanoparticles internalization

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Assessing the cellular response to external factors (exogeneous compounds, nanoparticles···) often require the use of microcroscopy techniques to visualize the internalization of these factors in living cells. In many studies, imaging using different probes is performed to provide complementary data by combining the advantages of each individual technique. This is particularly true in the frame of nanoparticle (NPs) internalization studies at the level of single cells. One of the main actual challenges is to track these NPs in single cells and assess the number of NPs internalized per cell. In the frame of such studies, we have developped a multimodal correlative microscopy (MCM) approach to detect, track, and quantify NPs in single cells. This MCM is based on the complementarity of three individual techniques: fluorescence microscopy (FM), scanning electron microscopy (SEM), and ion microbeam analysis (IBA) coherently focused on the same targeted individual cell cultured and maintained on a specifically designed sample holder. In this approach, a unique preparation protocol is applied and allows multimodal analysis of the same cell at different microscopes/microprobes. The correlated data obtained by FM and IBA open a field toward toxicological study on native NPs. Complementary results from SEM and IBA provide both surface and in depth information on the cell reaction as well as on the exact localization of NPs. Finally, IBA contributes to high-sensitivity and in situ quantification of all the present chemical elements including NPs in a single cell.
In addition to these methodological developments, we have completed the equipment of the CENBG nanobeam line to allow i) the use of two X-ray detectors combined to improve the detection solid angle and thus reduce the time required for analysis, ii) secondary electron imaging using our proton microbeam, iii) improve the scanning capabilities to increase the number of pixels per image.
The latest developments performed on the nanobeam as well as the application of MCM to nanoparticle quantification in single cells will be presented.

Primary author: Mr LE TREQUESSER, Quentin (University of Bordeaux, ICNCB - CNRS, Bordeaux, France)

Co-authors: Dr MICHELET, Claire (CENBG - University of Bordeaux , Bordeaux, France); Dr SAEZ, Gladys (CENBG - University of Bordeaux - CNRS, Bordeaux, France); Dr SEZNEC, Hervé (CENBG - University of Bordeaux - CNRS, Bordeaux, France); Mr DAUDIN, Laurent (CENBG - University of Bordeaux - CNRS, Bordeaux, France); Dr DELVILLE, Marie-Hélène (University of Bordeaux, ICNCB - CNRS, Bordeaux, France); Dr BARBERET, Philippe (CENBG - University of Bordeaux - CNRS, Bordeaux, France); Prof. MORETTO, Philippe (CENBG - University of Bordeaux - CNRS, Bordeaux, Franc); Dr DEVES, guillaume (CENBG - University of Bordeaux - CNRS, Bordeaux, France)

Presenter: Dr BARBERET, Philippe (CENBG - University of Bordeaux - CNRS, Bordeaux, France)

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