Single or large population cell analysis? Pitfalls and advantages of different strategies for elemental quantification in cells

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Keywords: (Synchrotron –based X-ray fluorescence microscopy, chemical imaging, elemental quantification, fluorescence chemosensor)

Noteworthy advances have been achieved in the field of single cell analysis, permitting to increase our understanding of cellular heterogeneity, and to determine the intracellular distribution of elements. On the other hand, it is well known that the cells have evolved to cooperate closely to form tissues and whole organisms, plants or animals, since in a multicellular organism single cells have to act an advance level of cooperation [1]. Therefore, the possibility to obtain information coming from cell population is of paramount importance. We recently developed a standard-less approach providing a complete characterization of whole single-cells by combining XRFM and atomic force microscopy (AFM). This method allows the quantification of the intracellular spatial distribution and total concentration of fundamental life elements constituting the molecules of living systems: carbon (C), nitrogen (N), and oxygen (O) and of light metals such as magnesium (Mg) and sodium (Na) [2]. Among these elements Mg constitutes a very interesting candidate to be used as parameter in comparing single cells with population analysis, due its importance in the cell homeostasis. To this aim we exploited the features of a new fluorescent dye DCHQ5 [3], able to quantify the total amount of intracellular Mg in a large populations of cells by traditional fluorimetric technique.
Results showed that the average intracellular Mg concentration found in a sample of 15 single cells by XRFM-AFM analysis (Figure 1 right) is of the same order of magnitude of that found in a population of 10^5 cells assessed by DCHQ5-assisted fluorimetric assay (Figure 1, left). This is not a trivial result since recent advancement in single-cell analysis clearly showed heterogeneity in cell population previously assumed to be identical [4], a very recent study showed a difference of two orders of magnitude in the concentration of intracellular TiO2-nanoparticles assessed in single cells or in cell population [5]. The example reported here for Mg can obviously be extended to many elements, including the fundamental life elements C, N and O (figure 2), and to trace elements, whose dysregulation in cells is often responsible of important diseases.

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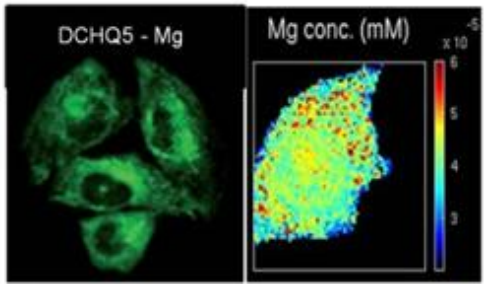

Figure 1 left, two-photon fluorescence microscopy images of LoVo cells stained with DCHQ5 probe. Right: Molar concentration map of intracellular Mg assessed by XRFM.

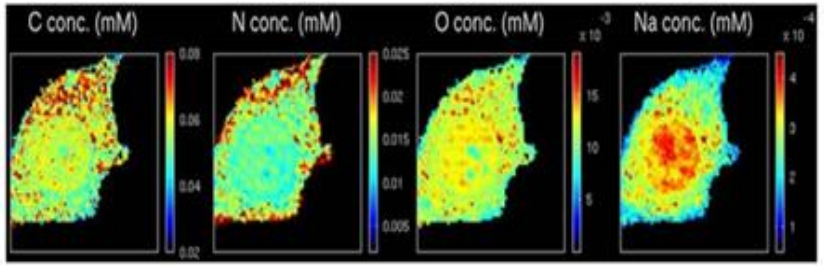

Figure 2 Molar concentration maps of intracellular C, N, O, Na assessed by XRFM combined with off-line AFM