Nanotomography and X-Ray Fluorescence Microscopy for quantitative Iron concentration map in inflamed cells

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Keywords: (Nanotomography, X-ray Fluorescence Microscopy, Intracellular Fe, Human THP-1 macrophages cells, Phase Contrast Imaging, Atomic Force Microscopy)

Iron is a primary component of fundamental processes in the cell. However, iron can become toxic when present in excess because of its capacity to don electrons to oxygen, thus causing the generation of reactive oxygen species, such as superoxide anions and hydroxyl radicals. In human fluids, free iron is maintained at 10^{-18}M concentration thanks to several proteins as lactoferrin (LF) in secretions and transferrin in blood. The altered iron balance favors bacterial infection and the related inflammatory response as occurs in cystic fibrosis [1,2]. Therefore, it is of great importance to provide quantitative mapping of iron concentration at high spatial resolution. Here we studied human phagocytic cells unstimulated or stimulated with bacterial lipopolysaccharide (LPS) or/and Lf to map the intracellular density and iron concentration. For this aim, X-ray fluorescence microscopy (XRFM), atomic force microscopy (AFM), X-ray phase contrast imaging and Phase Contrast Nanotomography were combined. In a recent paper [3, 4], we have demonstrated that it is possible to merge compositional and morphological information to quantitatively derive the element concentration combining XRFM with Atomic Force Microscopy (AFM). Here we aim to demonstrate that it is possible to quantitatively derive the elemental concentration combining XRFM with phase contrast nanotomography. The nanotomography is of paramount importance to reach the volumetric information in frozen-hydrated cells because AFM cannot be used since frozen hydrated cells are stored in liquid nitrogen. Combination of these techniques makes possible accurate description of compositional and structural cell features. The XRFM and phase contrast measurements 2D/3D have been carried out at the beamline ID16A-NI at ESRF, with the spatial resolutions of 100 nm and 50 nm, respectively; the volume of freeze-dried cells has been obtained by AFM with lateral resolution of 100 nm. To determine the concentration map we normalized the fluorescence intensity with the volume of the illuminated region (Fig.1). Moreover, we determined the weight fraction distribution map, normalizing the fluorescence intensity with the projected density obtained by phase contrast imaging (Fig.2) [5]. Indeed, we obtained the density distribution of the cells (Fig.3) by normalizing phase reconstruction maps with AFM data. Similar evaluations were carried out for LF- and LPS plus LF-treated cells. We also carried out nanotomography measurements, to obtain the iron three-dimensional density distribution (Fig.4). We succeeded for the first time in deriving quantitative concentration maps from combined use of XRFM and Phase Contrast nanotomography at nanometer scale spatial resolution (Fig.5). To obtain the thickness map from nanotomography we have not used the reconstructed tomographic image in terms of representation of the local electron density; instead, we have used it to obtain a morphological segmentation of the cell [6], and then we projected along the thinnest direction of this 3D mask. We obtained the thickness map from nanotomography by summing along the short axis and converting the pixel size into micrometres. It is worth to note that in the case of freeze dried cells the ratio between thickness and lateral dimension is very small, and this makes the quantitative derivation of cell thickness more difficult. In this work, we have demonstrated that it is possible to obtain the distribution map of the major cell components (as Iron, Phosphorus and Potassium), using XRFM, and the volume information obtained by phase contrast nanotomography or AFM. This work opens the way to quantitative biological analysis at nanometre spatial resolution using synchrotron radiation Imaging techniques. This makes possible to study of frozen hydrated cells as well, in case AFM cannot be used (Fig6).

The research was granted by Sapienza University of Rome to PV and FB and by a STSM of the Cost 1207.

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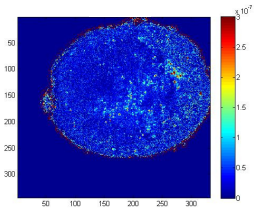

Figure 1. Iron concentration map [nM] of cell treated with LPS.

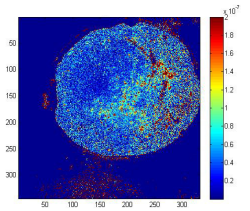

Figure 2. Iron Weight fraction distribution map of cell treated with LPS.

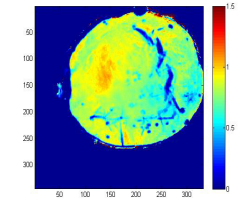

Figure 3. Density distribution map [g/cm^3] of cell treated with LPS.

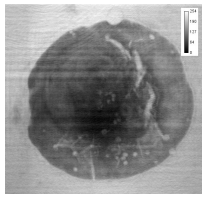

Figure 4. Density Map 3D from Nanotomography [g/cm^3] of cell treated with LPS.

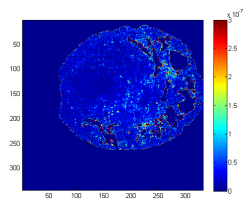

Figure 5. Iron Concentrations maps from Nanotomography [nM] of cell treated with LPS.

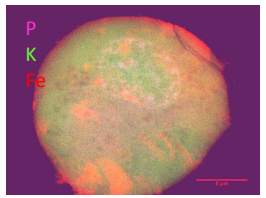

Figure 6. Fluorescence intensity Composite Map of frozen hydrated control cell.