Quantitative high resolution mapping of Fe concentration in inflamed cells, combining X-ray Fluorescence Microscopy, Phase Contrast Imaging and Atomic Force Microscopy

Chiara Gramaccioni[a], Alessandra Frioni[b], Andrea Notargiacomo[c], Michela Fratini[d], Emil Malucelli[e], Stefano Iotti[e,f], Yang Yang[g], Peter Cloetens[g], Lorenzo Massimi[h], Piera Valenti[b], Stefano Lagomarsino[i], and Francesca Berlutti[b].

[a]Dept. of Phys. Univ. of Cosenza, Arcavata di Rende (Cosenza), 87036, Italy
[b]Dept. of Public Health and Infectiuos Diseases Univ.Sapienza, Rome, 00185, Italy
[c]Institute for Photonics and Nanotechnologies - CNR, Rome, 00156, Italy
[d]Dipartimento di Scienze Univ.Roma Tre, Rome, 00154, Italy
[e]Dept. of Biotechnology and Pharmacy.Univ.Bologna, Bologna, 40127, Italy
[f],National Institute of Biostructure and Biosystem, Rome, 00136, Italy
[g]ESRF., Grenoble, 38043, France
[h]Dept. of Phys. Univ.Sapienza, Rome,00185, Italy
[i]IPCF-CNR, Rome, 00185, Italy
e-mail: chiara.gramaccioni@gmail.com

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Iron, an essential element for cell growth and proliferation, is a component of fundamental processes, such as DNA replication and energy production. However, iron can also be toxic when present in excess because of its capacity to donate electrons to oxygen, thus causing the generation of toxic reactive oxygen species. Therefore, 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. At the cellular level, iron is acquired via the binding of iron-saturated Tf to the transferrin receptors, in the cytoplasm is stored by ferritin or constituted the cytosolic iron pool, and is exported by ferroportin. The altered iron balance favors bacterial infection and the related inflammatory response as occurs in cystic fibrosis patients in which the high iron concentration in airway secretions positively correlates with bacterial infections and inflammation and negatively correlates with lung functionality [1] [2]. It is therefore of great importance to quantitatively map the iron concentration at high spatial resolution. In this study, murine phagocytic cells unstimulated or stimulated with bacterial Lyposaccharide (LPS) or/and Lf were analyzed to map and quantify cytosolic iron. X-ray fluorescence microscopy (XRFM) is one of the most powerful techniques to determine the element spatial distribution. Although, to quantitatively determine the concentration map, it is necessary to normalize the fluorescence intensity map with the volume of the illuminated region. In whole cells this can be done complementing the XRFM measurements with atomic force microscopy (AFM) measurements, as demonstrated in previous works [3,4]. Another approach is to determine the weight fraction distribution map, normalizing the fluorescence intensity with the projected density obtained by phase contrast imaging [5]. In this work we combined XRFM, AFM and phase contrast imaging (fig. 1), in order to have the maximum of compositional and structural information. Indeed, by normalizing phase reconstruction maps with AFM, it is possible to obtain the density distribution. We also carried out nanotomography measurements, to obtain the three-dimensional density distribution. The measurements have been carried out at the beamline ID16A-NI at ESRF, with spatial resolution of 100 nm for XRFM, and 50 nm for phase contrast imaging. AFM maps have been collected,with lateral spatial resolution of 100 nm and accuracy in the cell thickness measurement of few tens of nm. We obtain encouraging results combining these methods. The advantage of this new method is due to by the fact that Phase contrast imaging have the advantage that measurements can be carried out at the same time and on the same conditions as the XRFM, but the procedure to quantitatively reconstruct the phase variation is not straightforward, and non trivial reconstruction algorithms must be applied. AFM, on the contrary, directly measures the topography of the cell, thus allowing to directly obtain the concentration distribution, which is the quantity generally used to describe the bio-chemical reactions.

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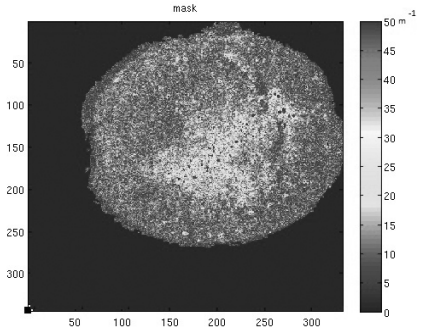

Figure 1. Fluorescence Maps for iron of an infected cell

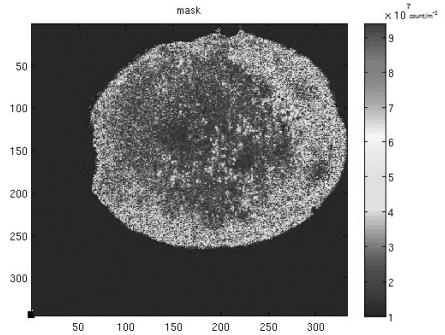

Figure 2. Fluorescence normalized with AFM topographic maps of an infected cell, proportional to iron concentration.

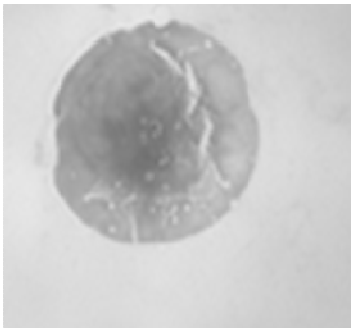

Figure 3. 2D Phase Reconstruction of an infected cell.