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

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Iron is a primary component of fundamental processes in the cell. In human fluids, free iron is maintained at 10^{-18M} concentration thanks to several proteins as lactoferrin (LF) [1]. 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 order to have an accurate description of compositional and structural cell features. 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. 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 (Figure 1). Moreover, we determined the weight fraction distribution map, normalizing the fluorescence intensity with the projected density obtained by phase contrast imaging [2]. Indeed, we obtained the density distribution of the cells by normalizing phase reconstruction maps with AFM data. 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. 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 [3], 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 (Figure 1A). This work opens the way to quantitative biological analysis at nanometre spatial resolution using synchrotron radiation Imaging techniques.

[1] P. Valenti et al., 2015, 30, 259
[2] E. Kosior et al.,. J. Struct. Biol. 2012 177(2):239-47
[3] Gramaccioni et al. Journal of Physics: Conference Series (JPCS), for the XRM2016 conference proceedings.

Fig. 1 Cell thickness map as obtained by a) holotomogrphy and b) AFM