EUROPEAN SYNCHROTRON RADIATION FACILITY
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Experiment Report Form

	Experiment title: Quantitative mapping of Fe concentration at nanoscale spatial resolution in bacterial injured epithelial cells	**Experiment number**: LS2362
Beamline: ID16A-NI	**Date of experiment**: from: 31th January 2015 to: 6th February 2015	**Date of report**: 21th April 2015
Shifts: 15	**Local contact(s)**: Peter Cloetens, Yang Yang	*Received at ESRF:*

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Report:

The main aim of this proposal was to quantitatively determine the map of iron concentration at nanoscale spatial resolution in epithelial cells infected by bacterial pathogens in the presence or absence of lactoferrin (Lf), an iron-chelating glycoprotein of natural immunity. With the experiment we expext to obtain useful information about the role of lactoferrin in iron homeostasis in infected cells. The samples were constituted of the murine cell line J774A.1 (ATCC® TIB-67™) treated with lipopolysaccharide (LPS) extracted from Escherichia coli 0111:B4 and with bovine lactoferrin.To this purpose we combined X-ray Fluorescence Microscopy (XRFM) measurements with x-ray phase contrast imaging in 2D, nanotomography and off-line Atomic Force Microscopy (AFM). The combination of all these techniques allow to quantitatively determine at nanometric spatial resolution compositional and morphological information.

The experiment run smoothly and we succeeded in obtaining fluorescence maps at 100 nm spatial resolution of several elements from P to Zn, including Iron that was the main target of the experiment. Phase contrast imaging at 50 nm spatial resolution in 2D was made on all the cells, before and after the fluorescence scans, in order to get information about possible radiation damage. Nanotomography at 50 and 25 nm resolution was also carried out on selected cells. Before the experiment session at ESRF, AFM was carried out on the same cells, in order to obtain independent information about morphology. All the measurements were repeated for untreated samples, cells treated with Lf, and infected cells with and without Lf. In the

following we show examples of fluorescence maps (Figure 1) and of 2D phase reconstruction (Figure 4)~~;~~ of an infected cell. Tomographic reconstruction is in progress. The experiment was made on freeze dried cells, because the cryogenic stage needed for performing measurements on frozen hydrated cells was not yet available.

The beamline run perfectly and the assistance from the staff was excellent.

We started the data analysis, which is quite complex: initially, we analyzed the fluorescence spectra with PyMCA, obtaining the integrated intensity of several fluorescence lines, including iron, for all the measured samples; in parallel we carried out phase reconstruction using the programs elaborated by the beamline staff, but obtaining reliable quantitative phase retrieval on all the samples requires further efforts. Moreover, we registered the fluorescence intensity maps with AFM topographic maps, obtaining concentration maps. When the phase retrieval procedure will be completed, we will be able to compare concentration maps with weight fraction maps. From previous work [1] we already know that the two maps generally do not coincide. Then we will analyze also the tomographic data, in order to have three dimensional structural information. We also made 2-D Phase Contrast imaging before and after fluorescence measurements, to monitor radiation damage. Slight differences were noticed between the two images. Radiation damage could be strongly alleviated measuring frozen hydrated cells. A new proposal has been made for this purpose.

In the following we show an example of the fluorescence intensity map (Fig. 1) of an infected cell, the AFM topographic map (Fig. 2), the fluorescence normalized with AFM, proportional to iron concentration (Fig. 3), and 2D phase reconstruction (Fig.4), of the same infected cell.

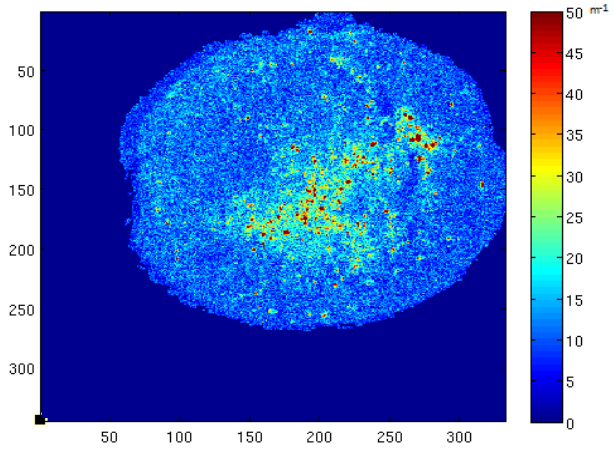


Figure 1. Fluorescence Maps for iron of an infected cell.

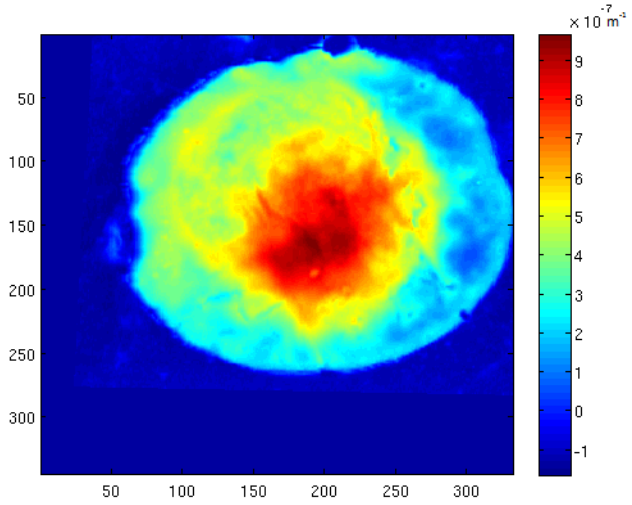
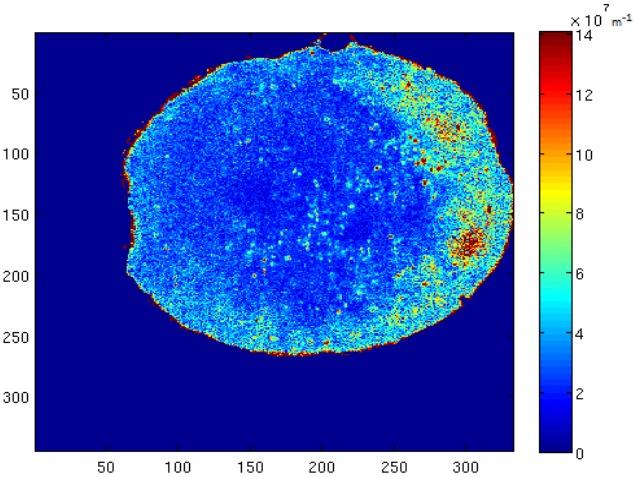


Figure2. AFM topographic maps aligned with the fluorescence map of the same cell



~~F~~**Figure3**. Iron Fluorescence normalized with AFM topographic

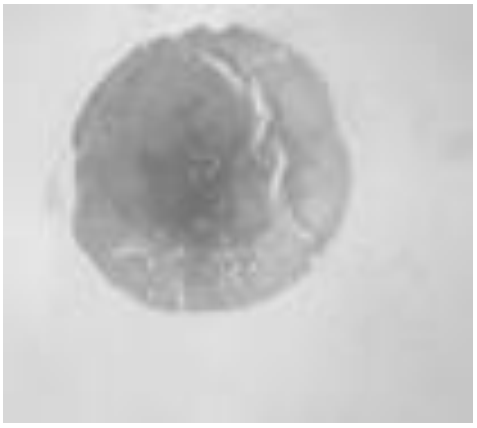


Figure4. 2D Phase Reconstruction of the same cell

map of the same cell, proportional to iron concentration.

[1] Malucelli E. et al., 2014. Anal. Chem, 86 5108-5115.