EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

ESRF	**Experiment title:** Quantitative mapping of Fe concentration at nanoscale spatial resolution in frozen hydrated bacterial injured human cells.	**Experiment number**: LS2551
Beamline: ID16A-NI	**Date of experiment**: from: 23th February 2017 to: 28th February 2017	**Date of report**: 5th April 2017
Shifts: 15	**Local contact(s)**:Yang Yang, Alexandra Joita pacureanu	*Received at ESRF:*

Names and affiliations of applicants:

Gramaccioni Chiara, Physics Department University of Calabria, Cosenza (Italy);
Lagomarsino Stefano, CNR - IPCF c/o Physics Department, University Sapienza, Rome (Italy);
Cloetens Peter, European Synchrotron Radiation Facility (ESRF), Grenoble, France;
Yang Yang , European Synchrotron Radiation Facility (ESRF), Grenoble, France;
Valenti Piera, Dept. of Public Health and infectious diseases, University Sapienza Rome (Italy);
Berlutti Francesca, Dept. of Public Health and infectious diseases, University Sapienza Rome (Italy);
Rosa Luigi, Dept. of Public Health and infectious diseases, University Sapienza Rome (Italy);
Frioni Alessandra, Dept. of Public Health and infectious diseases, University Sapienza Rome (Italy);
Malucelli Emil, University of Bologna Department of Pharmacy and BioTechnology, Bologna (Italy);
Procopio Alessandra, University of Bologna Department of Pharmacy and BioTechnology, Bologna (Italy).

Report:

This proposal is the continuation of proposals ls2433, carried out from 24th February to 29th February 2016 at BL ID16A-NI. The main aim of both proposals was to quantitatively determine the intracellular map of iron concentration at nanoscale spatial resolution in cells infected by bacterial pathogens in the presence or absence of lactoferrin, (Lf), an iron-chelating glycoprotein of natural immunity (Valenti & Antonini 2005, Berlutti et al. 2011).

The aim of the experiment ls2551 was to complete the experiment carried out before, by acquiring tomographic scans together with the fluorescence maps, in order to derive also concentration maps, the quantity of reference in the biological process.

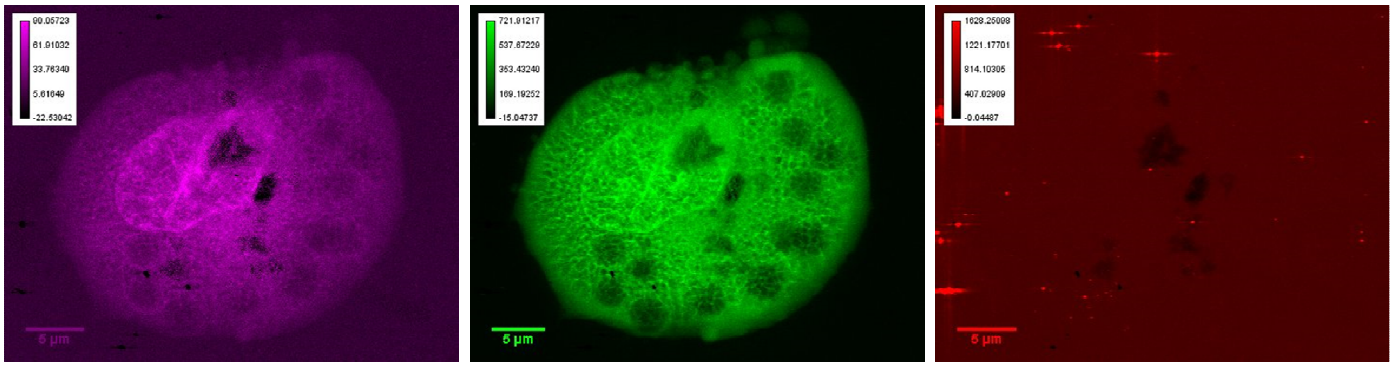
We have plan to analyze four different macrophage preparations: untreated cells, cells treated with lipopolysaccharide (LPS) or lactoferrin (Lf), and cells treated with LPS plus Lf. The experiment run smoothly and we succeeded in obtaining only on two different

macrophage preparations: LPS and Lps+Lf 3D tomographic scans together with the fluorescence maps at 120nm spatial resolution.

On the other two different macrophage preparations Lf and Control cells, we succeeded in obtaining Fluorescence maps at 70 nm spatial resolution of several elements from P to Zn, including Iron that was the main target of the experiment, Phase contrast imaging at 30 nm spatial resolution in 2D and Nanotomography at 40 nm resolution was also carried out on selected cells.

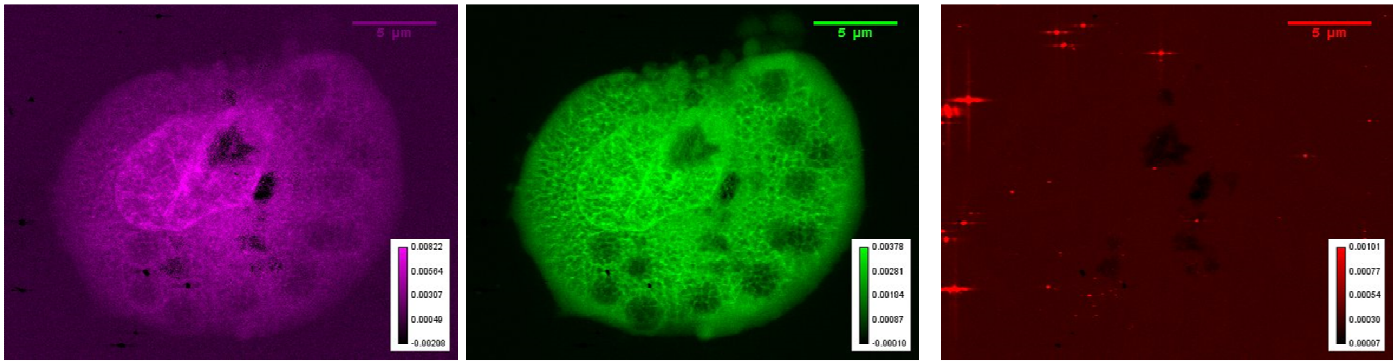
The beamline run perfectly and the assistance from the staff was excellent. We started the data analysis. Initially, we analyzed the fluorescence spectra with PyMCA, obtaining the integrated intensity of several fluorescence lines, including iron, for all the measured samples; in parallel we carried out phase reconstruction using the programs elaborated by the beamline staff. We carried out the reconstruction of 3D tomographic scans together with the fluorescence maps which is quite complex: we analyzed the fluorescence spectra, but we have to make the alignement with the tomographic scans in order to obtain the 3D Fluorescence tomogrpahy on the two samples investigated but it requires further efforts. A new proposal has been made ls2703 in order to finish the experiment ls2551, that can not end because of several falls Beam during the run. We submitted the new proposal ls2703 in order to complete 3D localization of the iron in untreated- (CTRL) and Lf-treated macrophages cells. This information will deepen the knowledge on the cell iron content and distribution as well as the Lf influence on intracellular iron concentration and distribution. The importance of Lf is witnessed by increasing scientific interest about its multiple functions as therapeutic agent of several human pathologies (see the website ClinicalTrials.gov where at now 55 clinical studies on lactoferrin are reported). Moreover, the Italian Group involved in present research, coordinated by prof Piera Valenti, member of International Scientific Committee on Lactoferrin is skilled on Lactoferrin (see the Special Issue of Biometals published after the XI International Conference on Lactoferrin Chair Prof. Piera Valenti, Rome 6-10 October 2013.). It is important to underline that the next XIII International Conference on Lactoferrin will be organized even by Prof.Piera Valenti, Rome November 2017.

In the following we show an example of the fluorescence intensity map reconstructed (Fig. 1) of an control cell, the Weight Fraction Distribution Map (Fig. 2) of the same cell.



A **B** **C**

Figure 1. Fluorescence Maps for: A Phosphore, B Potassium and C Iron of an infected cell (Control).



A **B** **C**

Figure 2. Weight Fraction Distribution Maps for: A Phosphore, B Potassium and C Iron of an infected cell (Control).