EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

	Experiment title: Quantifying and mapping the intracellular distribution of dye doped silica nanoparticles in colon cancer cells	**Experiment number**: LS-2491
Beamline: ID16A-NI	**Date of experiment**: from: 20th July 2016 to: 26th July 2016	**Date of report**: 09/12/2016
Shifts: 12	**Local contact(s)**: Alexandra Joita Pacureanu	*Received at ESRF:*

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Report:

The main aim of this proposal is to quantitatively determine at nanoscale the Si concentration and sub-organelles compartmentalization in frozen hydrated cancer cells loaded with dye doped silica nanoparticles (SiNPs). In the medical field multimodal imaging and theranostic tools represent the frontier research, and SiNPs are a great promise for the design and creation of inexpensive, hand-held kits. However, before this can occur, their accumulation and potential long-term toxicity has to be understood from a qualitative and quantitative point of view.

After a discussion with the beamline scientists, during the preparation of the experiments, we decided to move toward Fe nanoparticles (FeNPs) to maintain Si_3N_4 membranes as support needed for tomography experiment on frozen hydrated cells.

In this experiment, we used frozen hydrated LoVo cell loaded with dye doped FeNPs. LoVo cells were cultured in DMEM medium with FeNPs in the biological laboratory at the ESRF and then cryofixed using the plunge-freezer apparatus present @ the Id16 NI beamline. We measure frozen hydrated cells in order to examine nanoparticle intracellular incorporation as close as possible to their native state, and to decrease the radiation damage. Frozen hydrated LoVo were transfer in the microscope and measured under cryogenic condition.

We will choose colon cancer cells (LoVo) as they represent a consolidated model of tumor cell lines (level S1 according to directive 2000/54/EC)
We succeeded in recording phase contrast holotomography and fluorescnce tomography in 2 LoVo cells loaded with FeNP. The frozen hydrated cells analysed were covered with a an estimated thickness of ice ranging from 10 to 50 nm. The phase contrast holotomography has been acquired rotanting the sample over an angle of 180 degrees taking 1600 different projections at 4 different distances, with a pixel sixe of 50 nm. The fluorescnce tomography has been acquired taking 30 different fluorescences over an angle of 180 degrees with a pixel size of 150 nm.
The postprocessing analysis is still ongoing but from the preliminary data we found intracellular spot of Fe only in one cell (Figure1). We ascribe the Fe signal (figure 1) to agglomerations of nanoparticles sice the same spots are present in almost all the fluorescence maps acquired at different angles of rotation. Next step will be the recostruction of the entire fluorescnce tomography with the help of the beamline scintists that gave us an excellent assistance during the beamtime.

Figure 1 shows the Fe agglomerations in 4 adiacent maps acquired. Red potassium signal highlighting the whole cell and in green the Fe spots.