ESRF	**Experiment title: Single cell irradiations: cell inactivation induced by X-ray micro beam targeted cytoplasmic and nucleus irradiations.**	**Experiment number**: LS-1836
Beamline: ID 21	**Date of experiment**: from: 02/04/01 to: 07/04/01	**Date of report**: 25/07/2001
Shifts: 12	**Local contact(s)**: B. Fayard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

* FAYARD Barbara, ID21, ESRF, BP220, 38043 Grenoble

* SALOME Murielle, ID21, ESRF, BP220, 38043 Grenoble
SUSINI Jean, ID21, ESRF, BP220, 38043 Grenoble
ROBERT-NICOUD Michel, IAB, Faculté de Médecine, Domaine de la Merci, 38706 La Tronche
* BATTEUX Bernadette, IAB, Faculté de Médecine, Domaine de la Merci, 38706 La Tronche
ORTEGA Richard, CNRS UMR 5084, BP 120 Le Haut Vigneau, 33175 Gradignan

Report:

The aim of this preliminary experiment was to study the consequences of targeted irradiations on single cells. Classical irradiation protocols use macro beam irradiations and study the averaged consequences on a population of cells. Micro beam such the one produced at ID21 offers the possibility to study radiation damage at the single cell level. This is of great interest for fundamental studies on the biological damages produced by ionising radiations and can be further applied to radiotherapy and radioprotection.

On ID21, a Fresnel zone plate is used to focus the beam to a submicron spot. The difficulty of the experiment relies in the fact that the cell positionning under the beam must be very accurate (within a few microns since the cell nucleus size is 6-8 μm) and, unlike for standard samples, the X-ray beam can not be used for positionning to prevent cell damage before irradiation. The developped solution consists of a pre-alignement of the cells under an optical microscope. In order to be accurate and fast, an image acquisition interface and a mechanical interface have been developed. The first one allows to point individually the cells, the coordinates are read on coders and automatically saved to be used afterwards for the positionning under the beam. The second one permits the alignment of the translation axis on both the optical microscope and the X-ray microscope. This combination has lead to very good results with a positionning accuracy measured to be less than 2 microns -see an example on figure 1-.

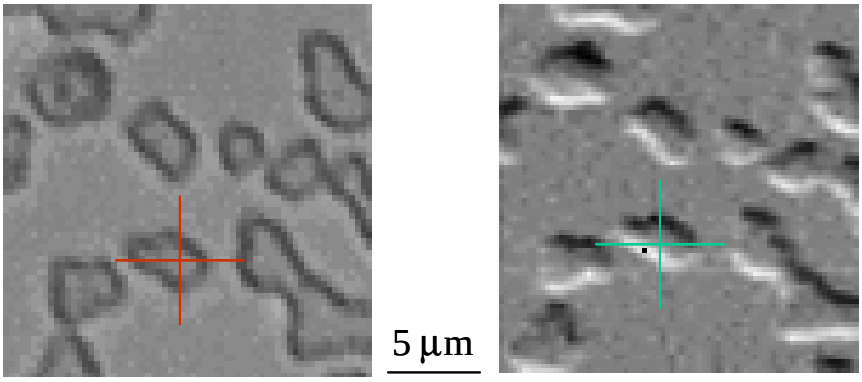

Figure1: left: optical image with cross on targeted point; right: X-ray image with cross on hit point. The test samples were aggregated polystyrene spheres with size smaller than cell nucleus size.

The second key point for irradiation experiment is the dosimetry. It is absolutely necessary to accurately measure the dose delivered to cells. The solution here consists in using a Io photodiode that measures the photon flux just before the sample. The set-up used was a holed photodiode coupled to a thin aluminium foil: the direct beam goes through the hole and the foil and the photodiode detects the fluorescence from the aluminium foil. This configuration was tested with a standard photodiode in place of the cells, to measure the direct intensity I. As shown in figure 2, I is directly proportionnal to Io with a standard deviation of 0.4%. This demonstrates that, after calibration of Io, this one can be used to monitor the dose delivered to the cells with a very good confidence.

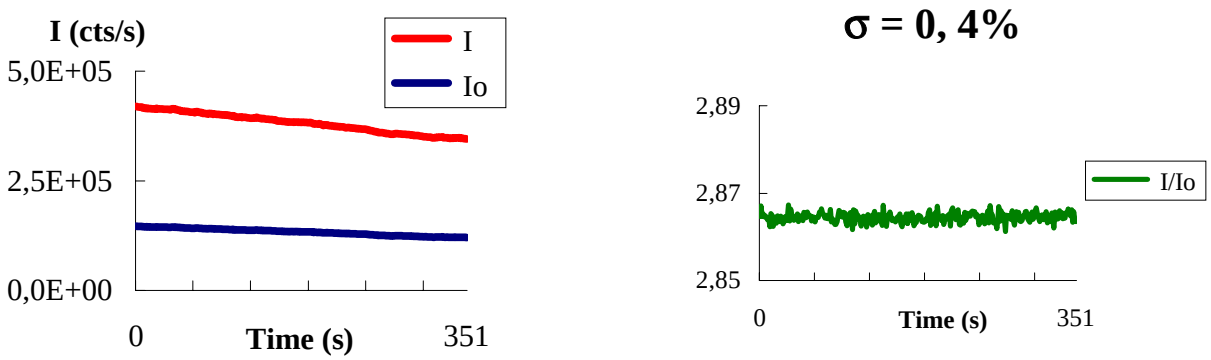

Figure 2: Left: time evolution of direct beam intensity (I) and fluorescence intensity (Io). Right: measured ratio between I and Io with σ, the measured standard deviation.

Conclusion:

The key points to realize micro-irradiations -positionning accuracy and dosimetry- have been extensively tested during this experiment. A very satisfying set-up has been reached. It combines a precise dose monitoring (<1%) and an accurate cell positionning under the beam (within 2 µm), both are near the experimental theoretical limits. After this feasability experiment, the next step is to perform micro-irradiations on living cells.