# Report for Experiment LS – 2339

TITLE: Correlation between the viscoelastic properties of healthy human cells submitted to synchrotron irradiation and their cytoskeletal deformation and adhesion properties

EXPERIMENTAL SESSION: ID17 – 04 October 2014/07 October 2014

Our aim is to study how mechanical properties of healthy human cells exposed or not to synchrotron X-Ray irradiation are modified in a dose dependent manner and how this can be linked to radiation-induced DNA lesions and radiosensitivity. For this purpose, we have decided at first to characterize these effects on 9L and F98 living cells in a pilot experiment, since these cell lines are known to show different radioresistance[1].

We have successfully tested the capabilities of our custom-made Atomic Force Microscope at ID17 imaging living cells (Figure 1).

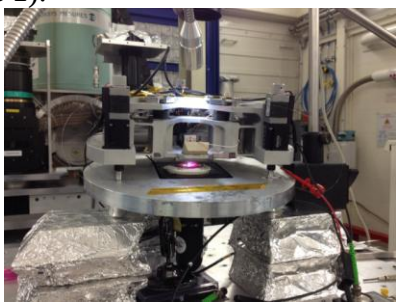

Figure 1: AFM mounted at ID17

Cells have been prepared at the Biomedical Facility on Thermanox plastic coverslips.
Cells have been imaged by AFM in DMEM, 1% PS in physiological conditions. The AFM cantilever used were Bruker MLCT, 0.01 N/m. We have characterized the cells in contact mode at constant force of 500 pN. The morphological study by AFM has shown consistent changes of the 9L cell morphology and cytoskeleton before and after irradiation (Figure 2).

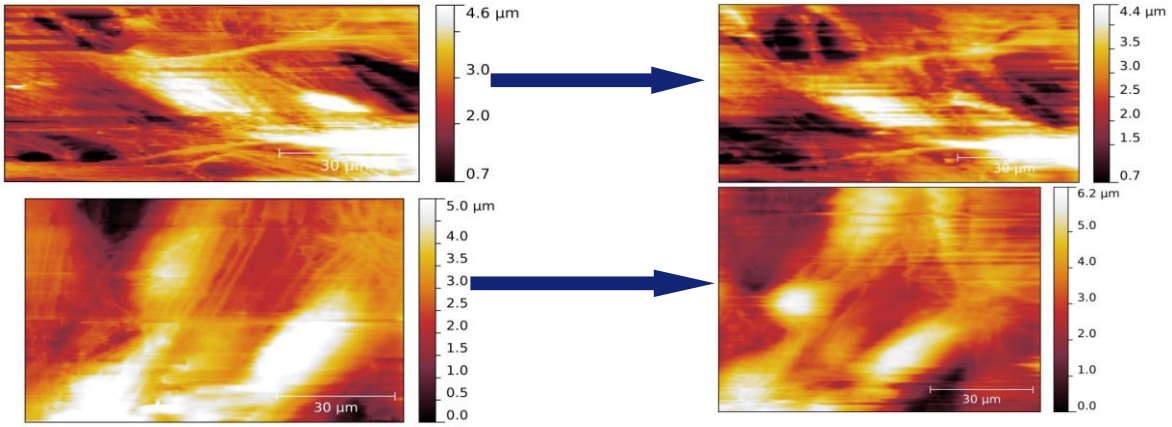


Figure 2: **top**: 9L cells imaged by AFM before (left) and after (right) irradiation of 3 Gy; **bottom**: repetition of the experiment on different 9L cells

F98 cells have been more challenging to be imaged by AFM since they have been observed to detach easily from the substrate. We aim to repeat the experiment on F98 cells employing fibronectin to ensure a more efficient fixation on the substrate.

The mechanical properties of the cells have been characterized through indentation experiments: at first the AFM probe enters in contact with the cell membrane, and then it starts to deform it by applying a force in the order of 100 pN – few nN. A typical graph is reported in Figure 3 where several parts of a cell have been indented, each corresponding to different colors. The Y-axes represents the measured force, while the X-axes represents the indentation length, hence the cell deformation. Since different parts of the cells have different elasticity (Figure 3), we have fixed a matrix of points on the cells and acquired several indentation curves (Figure 4): so-called AFM Force-Volume method.

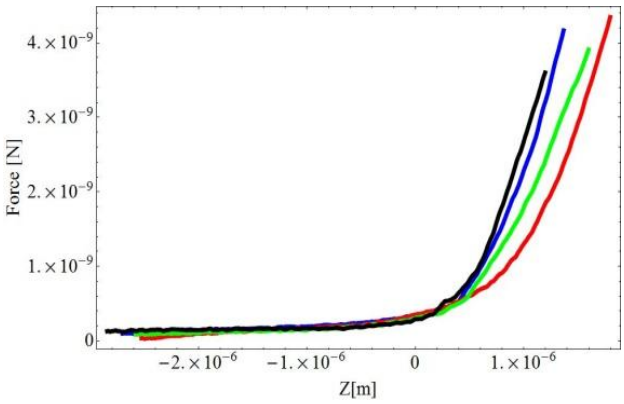


Figure 3: several indentation curves acquired on the very same living cell, showing that different parts of the cell have different elasticity

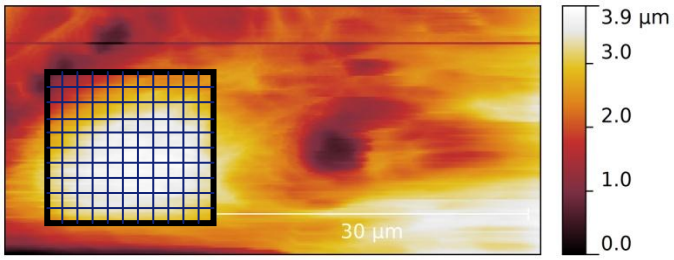


Figure 4: definition of a matrix of indentation points on a F98 living cell. An indentation curve will be acquired in each point.

We have evaluated the Young modulus of each indentation curve through the relationship between the force F and the indentation delta:

$$F = \frac{3Etan\theta}{4(1-\nu^2)}\delta^2$$

Where E is the cell Young modulus, nu its Poisson ration and theta the semi angle of the indenter, the AFM tip, here modeled as a cone. Once each indentation experiment was fitted, we have plot the distribution of the cell Young modulus as shown in figure 5 over typically 100 curves.

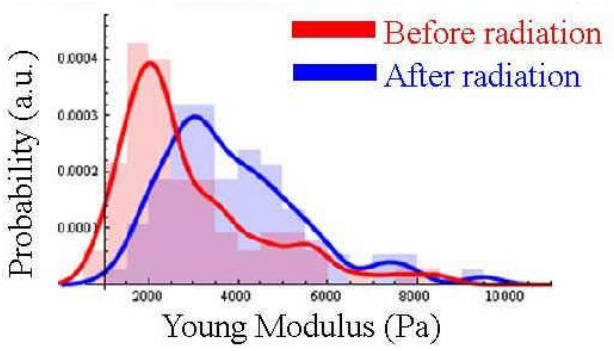


Figure 5: Elasticity measured over an entire 9L cell before (red) and after (blue) irradiation of 3 Gy

9L cells have been observed to show an overall increase of the cell stiffness 20 minutes after exposure to a dose of 3 Gy (exposure time in the order of the second).
Although the majority of the experiments performed during the 9 shifts have shown certain reproducibility, we have some exceptions. We will request for further beamtime in order to further evluate the reproducibility of this increase of the cell stiffness. We are convinced that the use of fibronectin, ensuring a better fixation of the cells on the substrate, will facilitate the acquisition of more reproducible AFM experiments, both from the morphological (especially on F98 cells) and mechanical point of view.

**References:**

1 Z. Bencokova et al., Molecular and cellular response of the most extensively used rodent glioma models to radiation and/or cisplatin, J.Neurooncol., 86, **(2008)**, pp. 13-21.