Adaption of the Cell Irradiation Setup at SNAKE for Live Cell Imaging

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For four years now the ion microprobe SNAKE is used to irradiate living cells with sub micrometer resolution [1,2]. One of the main aims in these experiments is to study the dynamics of proteins involved in the cellular response to DNA damage induced by energetic ions [3,4].

So far after irradiation the cells have been stored in the incubator for a defined time until they were fixed. After fixation the repair proteins of interest have been stained using immunofluorescence techniques. Thus the protein dispersion existent at the moment of fixation can be observed under the fluorescence microscope. But the observation of temporal protein activity within one cell is not possible using this procedure. In order to study the dynamics of repair proteins several cell samples have to be irradiated and processed after different time periods. The temporal protein behaviour can only be deduced from statistical analyses of the different samples.

But now for the first time it is possible to do live cell imaging at an ion microbeam. For this purpose the cells are no longer fixed and stained but they are genetically engineered in a way that they adhere fluorescent proteins (e. g. GFP, green fluorescent protein) to the repair proteins of interest. This allows to study the protein dispersion in the living cell at arbitrary points in time and investigate movements directly.

For this live cell imaging the irradiation setup at SNAKE has been modified in some ways. A high quality fluorescence microscope (Zeiss Axiovert 200M) is mounted to the beam line. Also phase contrast microscopy (e. g. for targeting purposes) is implemented (see fig. 1). The microscope stage, where the cell containers are mounted, the objective and the beam exit nozzle are all heated to 37° C. To keep the cells alive for a long time period, the cells are now surrounded by cell culture medium. In order to avoid a significant degradation of beam resolution, the exit nozzle is moved so close to the cells that the beam traverses not more than $30\mu m$ of culture medium during irradiation.

The main changes in the irradiation setup have been made to the ion detection, which is necessary for the single ion preparation [2]. The way to nurture the cells on a mylar foil and to detect the transmitted ions behind this foil is no more acceptable, because high quality microscopy requires for optical reasons, that the cells grow on a $170\,\mu m$ cover slip, which would stop the ions before they reach the detector. Beam detection in transmission before the cell sample is not tolerable, too, as this would corrupt the beam resolution. For these reasons the cells grow in the new setup on a $170\,\mu m$ plastic scintillator, which has a refraction index near to glass. So during irradiation every ion excites a flash of scintillation light, which is guided through the optical path of the microscope to a PMT mounted to one of the camera ports (see fig. 2).

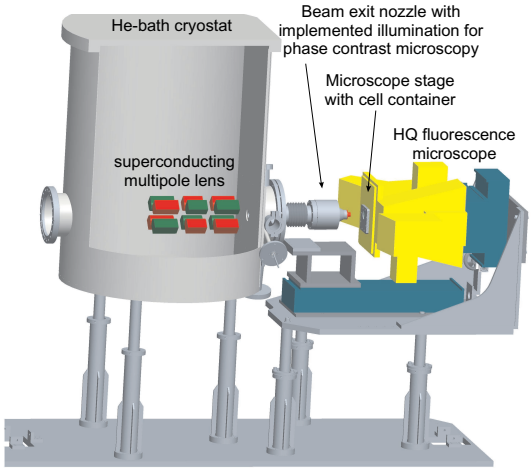

Fig. 1: CAD drawing of SNAKE live cell setup.

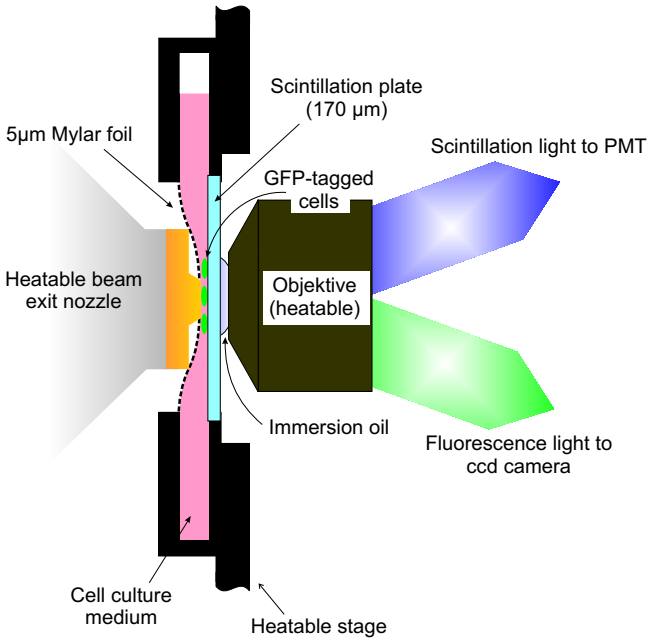

Fig. 2: Schematic setup for live cell irradiation and imaging.

After the irradiation it is possible to switch to the port where a CCD camera is mounted within about half a second and start microscopy of the living cells. As the scintillation plate is illuminated during microscopy, too, it is possible that it gets excited. To avoid interference between the scintillation light and the GFP signals a BC 418 is chosen as scintillator, as it emits light in the near UV. First time series of the repair proteins Rad 52 and Mdc1 in the living cell have already been taken using this new setup.

References

[1] Annual report 2003, p. 60.
[2] A. Hauptner *et al.*, Radiat Environ Biophys **42** (2004) 237-245
[3] Annual report 2004, p. 58.
[4] V. Hable *et al.*, Nucl. Instr. and Meth. **B245** (2006) 298-301