First Cell Irradiation Experiments with Microscopic Resolution at SNAKE

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During the year 2003 the setup for irradiating living cells at the ion microprobe SNAKE [1,2,3] started its operation successfully. This device is not only attractive to biological irradiation experiments due to the microscopical resolution enabling the selective irradiation of specific parts in a living cell like the cell nucleus or even nucleolus as a substructure of the nucleus. It also allows the irradiation with a broad range of different ion species available from the 14 MV tandem accelerator which highlights SNAKE as an irradiation tool among most other ion microprobe setups. In fig.1 the LET (=linear energy transfer) value as well as the absolute deposited energy in a water equivalent cell nucleus are plotted against ion energy per nucleon respectively ion velocity for a single ion hit.

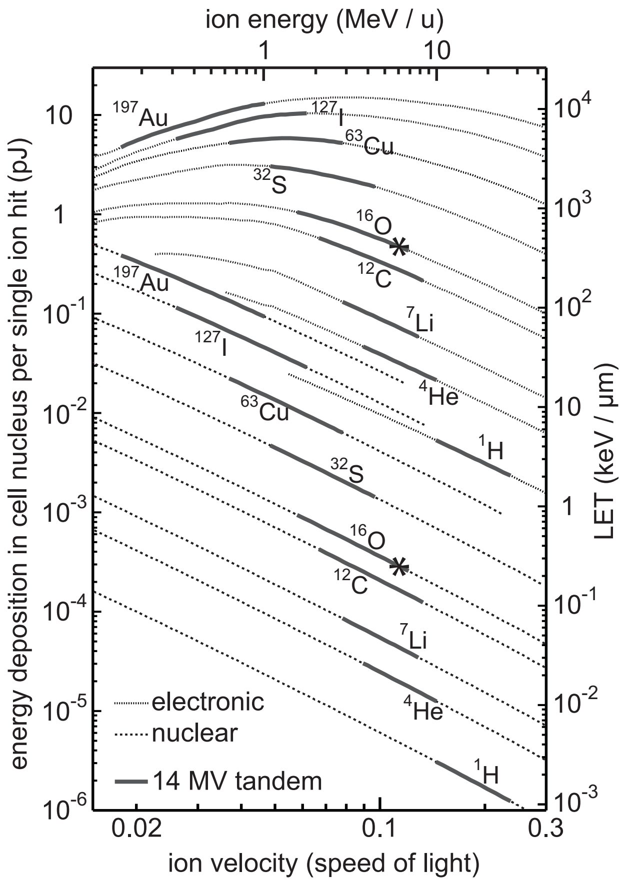

Fig. 1: LET and absolute energy deposition in a cell nucleus (7.15 μm diameter assumed) per single ion hit.

For each ion species the electronic (dotted lines) and nuclear (dashed lines) stopping are plotted separately. The solid line sections indicate the available energy regions at SNAKE. Also indicated in fig.1 by an asterisk is the position of 100 MeV ^{16}O ions depositing each an energy of 0.46 pJ in a cell nucleus. This beam already used in first experiments can be estimated to generate about 23 double strand breaks (=DSBs) in the DNA molecule of a living cell per single hit [3]. For the irradiation experiments the cells were grown on a 6 μm Mylar foil in a specially designed container. Then this container was sealed by another mylar foil and put upright into the focal plane of the horizontal focussed ion beam of SNAKE (fig.2).

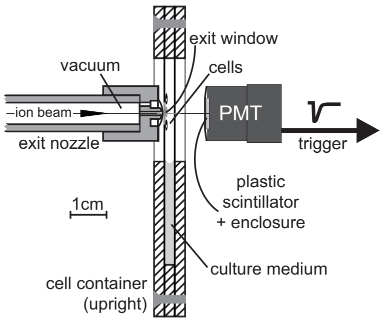

Fig. 2: Cell container in irradiation position.

The ion beam enters the atmosphere through an exit nozzle with a window (7.5 μm Kapton foil) keeping vacuum inside the beam tube system. Single ion detection is done by an assembly of a plastic scintillator, a small photomultiplier tube (PMT) and a light impervious foil. The ions therefore traverse the exit window, the mylar foils of the container, the cell sample and are finally stopped in the detector.

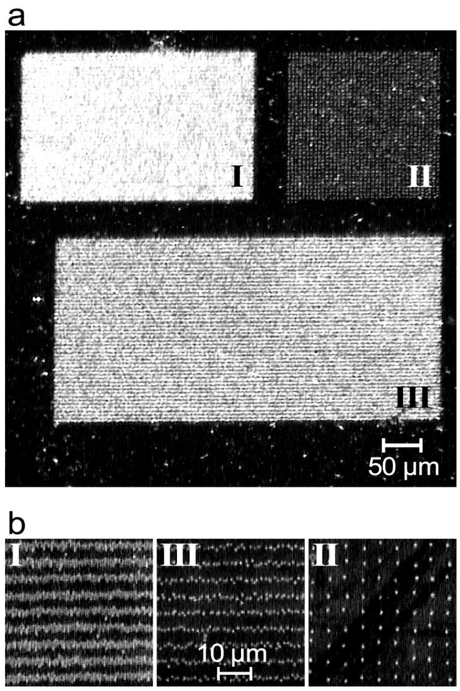

Fig. 3: Irradiation pattern in nuclear track detector.

Before a cell irradiation session takes place the ion beam is prepared and focussed using a scintillating CsI(Tl) screen observed by the adapted optical microscope [3] which also allows to look at the cells in the irradiation container.

In first experiments HeLa cells were irradiated with fixed patterns of counted 100 MeV ^{16}O ions. To ensure proper operation of the setup including beam scanning unit and single ion preparation the irradiated patterns were verified using polycarbonate foils (Makrofol) as nuclear track detectors. After an etching process the tracks of individual ions are visible in an optical microscope. A micrograph of the tripartite pattern is presented in fig.3a, cut-outs of the three sub-patterns at higher resolution in fig.3b (I-III). The matrix pattern (II) with a pitch of 5 μm was used to investigate the irradiation accuracy. Fig.4 shows the statistical evaluation of the deviations from the ideal position for a number of irradiation points. A resolution of 0.91 μm in x- and 1.6 μm in y-direction thus can be deduced.

After the cell irradiation the samples were incubated for one hour. During this time irradiation induced DSBs in the DNA are repaired by accumulation of the repair protein Rad51. A complex biochemical procedure [3] marks the protein Rad51 by indirect immunofluorescence staining which then can be observed using an epifluorescence microscope. In fig.5a,c a couple of cells acting as biological track detector shows the irradiated line pattern (III) as bright spots (foci) consisting of accumulated Rad51 protein. The grey shaded regions in the background represent the cell nuclei stained by another fluorescence dye. Obviously some cell nuclei performed an in-plane rotation after the irradiation. A micrograph of the "classical" nuclear track detector showing the pattern (III) in the same scale also is visible in fig.5b.

The structure of a single ion hit in greater detail can be seen in fig.6, where two cells (fig.6a,c) show the irradiated matrix pattern (II) (fig.6b, same scale).

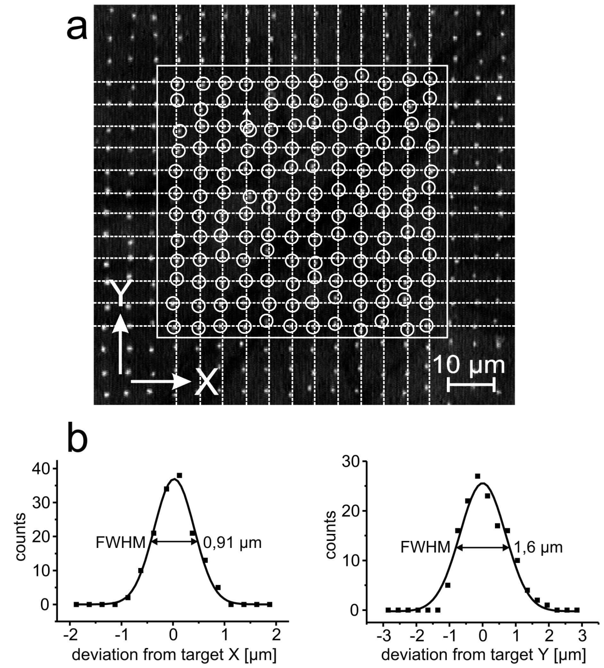

Fig. 4: Irradiation resolution determined by nuclear track detector.

There the accumulation of Rad51 exhibits significant split foci, which are not caused by accidental double ion hits at one point. Probably this indicates a movement of DNA material inside the cell nucleus during 1 hour DSB repair time [3].

References

[1] A. Hauptner *et al.*, Annual report 2002, p. 96
[2] A. Hauptner *et al.*, Rad. Res. **161** (2004) 98
[3] A. Hauptner *et al.*, Radiat Environ Biophys **42** (2004) 237-245

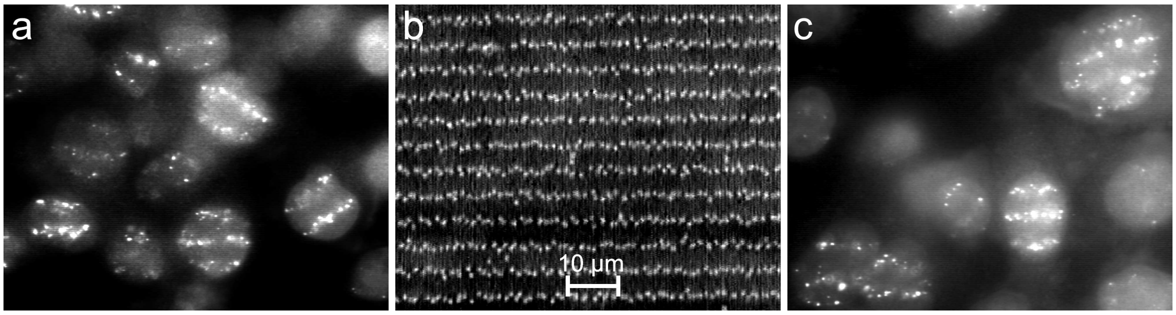

Fig. 5: a,c: Rad51 foci in HeLa cells after line irradiation. b: nuclear track detector (same scale).

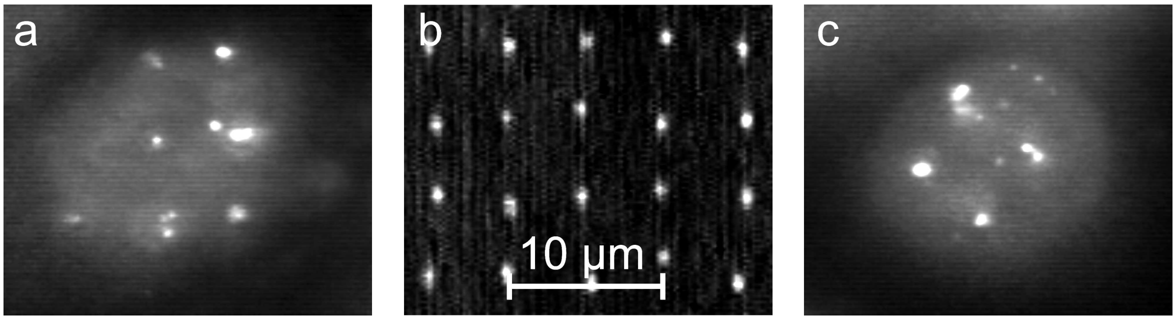

Fig. 6: a,c: Rad51 foci in HeLa cells after matrix irradiation. b: nuclear track detector (same scale).