# Evaluation of Protein Kinetics after Live Cell Irradiation and Imaging

V. Hable [a], T. Brüning [a], H. Strickfaden [b], G. A. Drexler [c], C. Greubel [a], G. Du, A. Hauptner, S. Koschartz, R. Krücken, S. Dietzel [b], T. Cremer [b], A. A. Friedl [c], and G. Dollinger [a]

[a] Angewandte Physik und Messtechnik, UniBw München [b] Department Biologie II, LMU-München
[c] Institut für Zellbiologie, LMU-München

The live cell imaging (LCI) facility at the ion microprobe SNAKE allows online studies of repair protein dispersion in living cells after heavy ion irradiation [1,2]. Here we describe the quantitative evaluation of protein kinetics from microirradiation experiments performed at the SNAKE LCI setup. The protein kinetics of interest here is the temporal process of repair proteins clustering to DNA damage sites caused by ion irradiation. These clusters are called foci.

The upper half of fig. 2 shows a series of fluorescence micrographs of a cell nucleus starting immediately after linewise irradiation with 55 MeV carbon ions. The repair protein Mdc1 is made visible by tagging it by the fluorescence protein GFP. As the repair protein is spread all around the cell nucleus, the morphology of the nucleus is visible under the fluorescence microscope before irradiation. Just a few seconds after irradiation foci formation can be observed. Quantitative evaluation of protein kinetics is done considering:

- Cell movements
- Foci movements within the cell nucleus
- Spontaneous foci not caused by irradiation
- Low signal to (cell-)background ratio
- Bleaching of the fluorescence proteins with time caused by illumination

First of all the algorithm developed for this analysis generates a new time series with cell movements and rotations being eliminated. Images of this series are pairwise subtracted (e.g. image 5 – image 1, image 6 – image 2 and so on). This process reveals pixels that have changed with time. These pixels are added to the region of interest (ROI, see second row of fig. 2), which represents a total area covering all foci in all time frames.

The protein concentration within the foci sites, which is assumed to be proportional to the brightness of the fluorescence signal, is evaluated versus time by determining the mean grey value $I_{foci}$ per pixel for the ROI. This value, however, is also affected by bleaching, which has an even stronger effect than the protein acuumulation at damage sites. So this value will decrease with time although the protein concentration increases within the ROI. To compensate for this the mean grey value $I_{control}$ in a second area, which is not affected by irradiation, is evaluated. The protein accumulation is measured by using the relative intensity $I_{rel} = I_{foci}/I_{control}$.

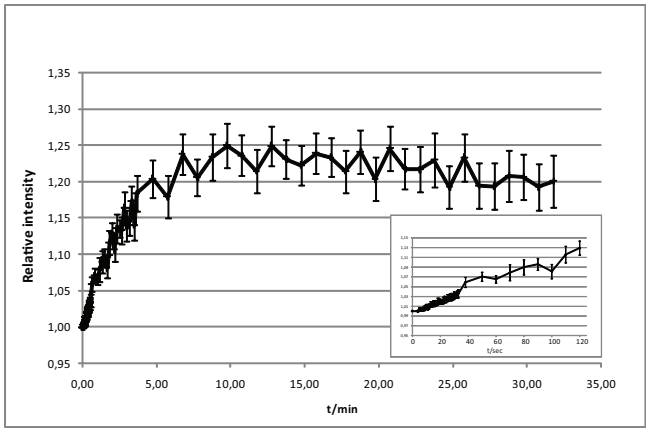


Fig. 1: Kinetics of repair protein Mdc1 in cells irradiated with 55 MeV carbon ions. The inlay shows the first 2 min.

The temporal development of this relative intensity is shown in fig. 1 for the average of a sample of five cell nuclei. One can see a fast increase of intensity already a few seconds after irradiation. After a few minutes the intensity maximum is reached and a much slower decrease of protein accumulation can be observed. This may be due to successful repair of some of the irradiation induced DNA damages.

## References

[1] Annual report 2006, p. 84.
[2] V. Hable *et al.*, Nucl. Instr. and Meth. B, accepted.

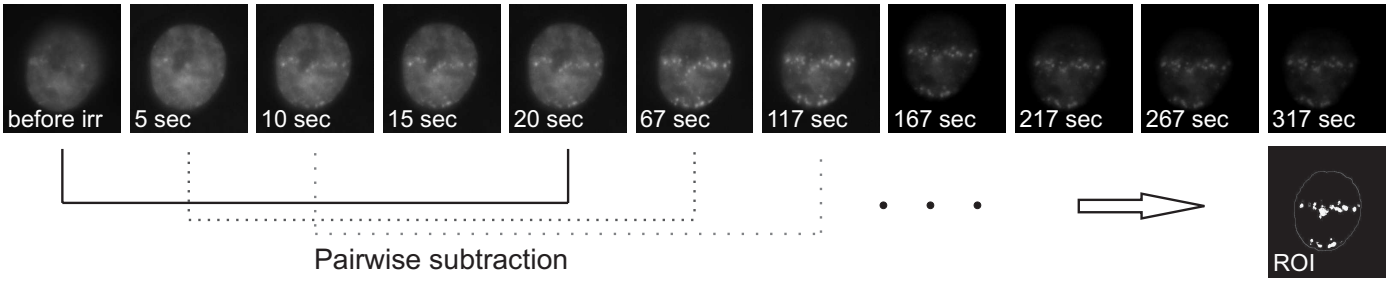


Fig. 2: Parts of a time series of a cell nucleus (diameter about 10 microns) with GFP tagged repair protein Mdc1. Time data relate to the irradiation moment. Already a few seconds after irradiation foci formation can be observed. Due to bleaching effects the images get darker with time. Pairwise subtraction of the images reveals areas where foci are formed. The merge of these areas results in the region of interest (ROI, white marked) in which the foci brightness is evluated.