Study of DNA–Damage Structure along the Tracks of Energetic Ions

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The cell irradiation setup at the scanning ion microprobe SNAKE is routinely used for biological microirradiation experiments [1]. In these experiments, DNA–repair factors accumulating at sites of radiation–induced DNA double–strand breaks are visualized using immunofluorescence techniques [2].

In 2005 new studies concerning the damage structure in cell nuclei along the tracks of single ions were started [3]. In order to obtain a high optical resolution along the track direction during fluorescence microscopy it is advantageous to perform the irradiation with a beam direction that is almost parallel to the cell carrier substrate [4,5]. Therefore HeLa–cells were irradiated at the irradiation setup of SNAKE in a modified geometry (see figure 1).

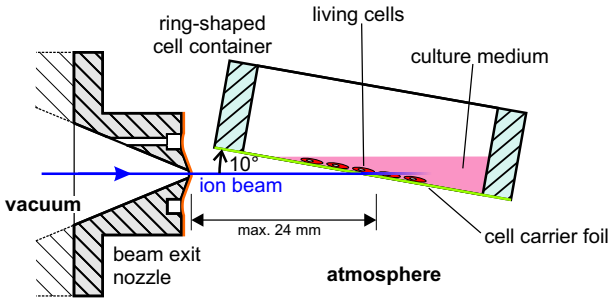

Fig. 1: Modified irradiation geometry: ion beam direction and cell carrier foil enclose an angle of 10°.

Due to the distance of several tens of millimeters between exit window and cells it is not possible to perform the irradiation with microscopic resolution. Furthermore, with this setup it is not possible to detect the ions that have transversed the cell sample. Thus, the particle fluence during irradiation was chosen to ascertain that in many cell nuclei single ion trajectories could be expected.

In figure 2 and 3 examples of HeLa–cell nuclei irradiated under the described geometry with each one particle are shown.

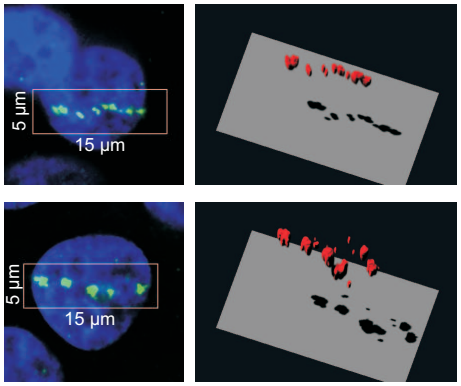

Fig. 2: Two tracks of single 29 MeV ^{7}Li–ions (LET = 86 keV/μm) in HeLa–cell nuclei.

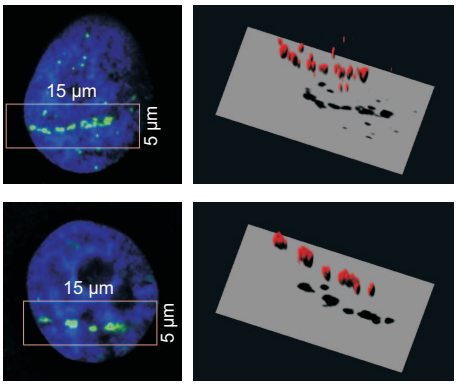

Fig. 3: Two tracks of single 24 MeV ^{12}C–ions (LET = 525 keV/μm) in HeLa–cell nuclei.

15 min after irradiation the cells were fixed and then immunostained for detection of the repair factor 53BP1. Z–image stacks were taken with an epifluorescence microscope. On the left side of figure 2 and 3 one can see a cross section through the cell nucleus containing the ion trajectory. Introducing an intensity threshold and using rendering software a three–dimensional reconstruction of ion tracks could be performed. The corresponding visualization is shown on the right side.

As a first result, in spite of identical irradiation conditions a variable appearance of the damage structure is evident, consisting of fine–grained foci on one hand (upper part) and of clustered structures on the other hand (lower part). Secondly, one notices a remarkable similarity between tracks of 29 MeV ^{7}Li– (figure 2) and 24 MeV ^{12}C–ions (figure 3). The corresponding LET (= linear energy transfer)–values differ by a factor of 6, but this is not reflected in the damage structures. It is possible that the distribution of DNA–damage sites is already saturated in the 29 MeV ^{7}Li–tracks with a LET–value of 86 keV/μm and that this reflects the architecture of the chromatin inside the cell nucleus.

From the fluorescence–images the density of DNA–damage foci can be roughly estimated to lie below 1 μm^{-1} along the particle track. In contrast, a simple model calculation assuming a cell nuclear architecture consisting of randomly distributed 30–nm–chromatin fibers suggests a foci density of about 1.8 μm^{-1} [3]. Recent calculations done at the Institute of Radiation Protection (GSF, Neuherberg) with the Monte–Carlo–Simulation–Code PARTRAC [6] predict an even higher foci density for the 24 MeV ^{12}C–tracks. The quantitative evaluation of experimental track structures and further comparisons with theoretical modelling are subject for future studies.

References

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