Improvements of the GSI microprobe for the targeted irradiation of single cells*

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Since the first successful targeted cell-irradiation in 2003 the GSI heavy ion microprobe has been shown to be a useful tool for studies in radiation biology [1, 2]. To further enhance its performance several technical improvements have been installed in the past year.

A problem affecting the aiming precision were variations in temperature which caused thermal expansions of the setup and generated slowly increasing targeting inaccuracies, forcing a frequent readjustment of the irradiation settings. To prevent this, the irradiation area was temperature stabilised. For an additional thermal stabilisation the microscope lamp was thermally isolated and the setup is ventilated by a temperature controlled fan which blows cool pre-filtered air into the system. The filtering of the air also enhances the sterility of the irradiation area. In tests over several hours the formerly observed displacements between beamline and optics could be decreased by an order of magnitude with this installation.

A method for a fast online aiming verification has been developed that allows us to test the beam deflection precision over the whole optical field of view. For that, a thin copper micro-grid and an energy resolving particle detector are placed at the sample position as shown in figure 1. After an automatic recognition, single ions are targeted onto the bars of the grid. All well targeted particles have to traverse the copper and reach the detector with reduced energy while misplaced ions reach the detector with full energy. By plotting the coordinates of the ions with full energies, the misaligned area can easily be visualised.

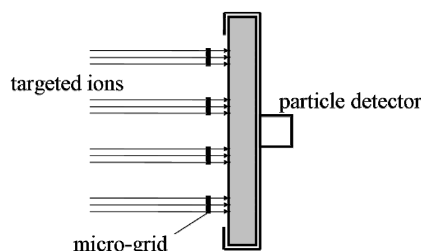

Fig. 1 Schematic setup of the online hit verification.

To test the targeting precision inside the cells, the ion tracks have been visualised by immunocytochemical staining of DNA damage response proteins [2]. To distinguish tracks from background foci, the ions were placed in cross patterns in which the single points were produced by one or twenty ions, respectively (see fig. 2). A comparison of the spots' sizes allows estimating the beams focal width. While the 1-hit spots have an average diameter of about 1.1 µm, the spot size increases for the 20-hit spots to roughly 1.8 µm. From these data one can conclude that all the 20 ions per spot have been placed in an area with a diameter of about 1µm.

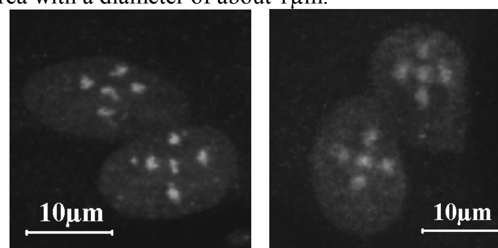

Fig 2: Cell nuclei irradiated with cross-patterns of C-ions with 4.8 MeV/u (5 points, 3 µm between the points) and stained for the damage response protein 53BP1. Left image: 1 hit/spot, right image: 20 hits/spot.

Regarding the biological application, the quantification of proteins involved in the cellular radiation response using immunofluorescence detection requires identical staining conditions. In order to control for this, special chambers were developed allowing the simultaneous staining of directly targeted cells, surrounding bystander cells and control cells. The control cells are completely separated from targeted and bystander cells by a stick in the middle of the chamber. Using this tool for targeted carbon irradiation, the analysis of the cell cycle regulating protein CDKN1A indicates a slightly increased CDKN1A induction in bystander cells (see fig. 3) confirming previous results obtained for low fluencies of carbon ions [4].

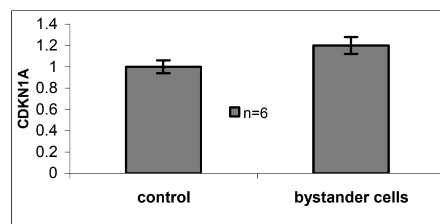

Fig 3: CDKN1A protein level in control and bystander cells following exposure to carbon ions (5 hits per nucleus, 3h after exposure)

References

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