

# DNA damage and repair kinetics after MRT emulation in living cells using monoenergetic synchrotron X-ray microbeams

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A novel, synchrotron-based approach, known as microbeam radiotherapy (MRT), currently shows considerable promise in increased tumour control and reduced normal tissue damage compared to conventional radiotherapy. We investigated different microbeam widths and separations using a controlled cell culture system and monoenergetic (5.35 keV) synchrotron X-rays in order to gain further insight into the underlying cellular response to MRT. DNA damage and repair was measured using fluorescent antibodies against phosphorylated histone H2AX ( $\gamma$ H2AX), which also allowed us to verify the exact location of the microbeam path. Beam dimensions that reproduced promising MRT strategies were used to identify useful methods to study the underpinnings of MRT. These studies include the investigation of different spatial configurations on bystander effects.  $\gamma$ H2AX foci were robustly induced by 1 hour in directly hit cells. Considerable DNA double-strand break repair occurred by 12 hours post-10 Gy irradiation, however, many cells still retained a few  $\gamma$ H2AX foci at the 12 hr time point.  $\gamma$ H2AX foci at later time points did not directly correspond with the targeted regions suggesting cell movement or bystander effects as potential mechanism for MRT effectiveness. In other investigations, irradiation of the cytoplasm only did not reveal any foci 1 hour post-radiation. Partial irradiation of single nuclei was also investigated and in most cases  $\gamma$ H2AX foci were not observed outside the field of irradiation within one hour after irradiation, indicating very little chromatin movement in this time frame. These studies contribute to the understanding of the fundamental radiation biology relating to the MRT response, a potential new therapy for cancer patients.