IMAGING OF DNA DOUBLE-STRAND BREAKS BY SUPER-RESOLUTION NANOSCOPY

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Genomic DNA is continuously being damaged by both exogenous (including non-ionising and ionising radiation) and endogenous agents. DNA double strand break (DSB) formation triggers the activation of many factors, including the phosphorylation of the histone variant H2AX, producing gamma-H2AX (γH2AX), which plays a crucial role in the DNA damage response. Repair protein Ku is involved in the first stage of DNA DSB recognition and acts as a scaffold for other repair proteins. Tumour suppressor p53-binding protein 1 (53BP1) localizes rapidly to sites of DNA DSBs. γH2AX, 53BP1 and Ku assays are well suited for studying the induction and repair of DSBs. The number of γH2AX ionising radiation-induced foci has previously been shown to be proportional to the amount of DSB produced. However, there is some discrepancy in the foci yield between different techniques such as gel electrophoresis and imaging. Here we investigate selected super-resolution techniques (ground-state depletion microscopy followed by individual molecule return (GSDIM), stimulated emission depletion (STED), structured illumination microscopy (SIM), in addition to an improved confocal, Airyscan and HyVolution 2) to study the differences in spatial distribution and amount of γH2AX foci formation after X-ray irradiation[1]. We show that using super-resolution techniques (down to 30-nm resolution) instead of standard microscopy reveals an increased number of foci per radiation dose (see Figure 1). Proteins 53BP1 (after hard X-ray irradiation) and Ku70/Ku80 (after laser microbeam irradiation) do not always yield a significantly higher foci number when imaged using super-resolution methods, suggesting that γH2AX, 53BP1 and Ku70/Ku80 DNA repair proteins do not fully co-localize on the units of higher orders of DNA packing. Unlike X-rays which deposit energy along the resulting electron tracks, alpha-particles produce a high density of ionisation events along their track and are referred to as high LET (linear energy transfer) particles. We have initiated the investigation of foci formation following high LET irradiation using super-resolution techniques. Preliminary data shows that high LET-induced foci are not resolved to the same resolution as low LET-induced foci.

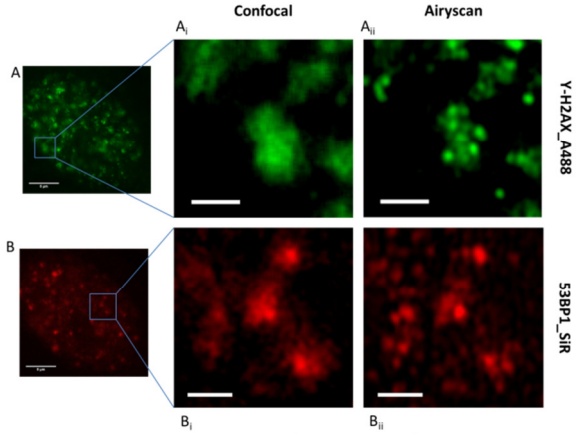

Figure 1. HeLa cells irradiated with 2 Gy hard X-rays followed by immunolabelling with γ-H2AX-Alexa488 or 53BP1-SiR (silicon rhodamine). Scale bars A , B: 5 µm; Ai, Aii, Bi , Bii: 1 µm.

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[1] S. D'Abrantes, *et al.* "Super-Resolution Nanoscopy Imaging Applied to DNA Double-Strand Breaks," *Radiation Research*, **189**, 19-31 (2018).