

A novel approach to double-strand DNA break analysis through γ -H2AX confocal image quantification and bio-dosimetry

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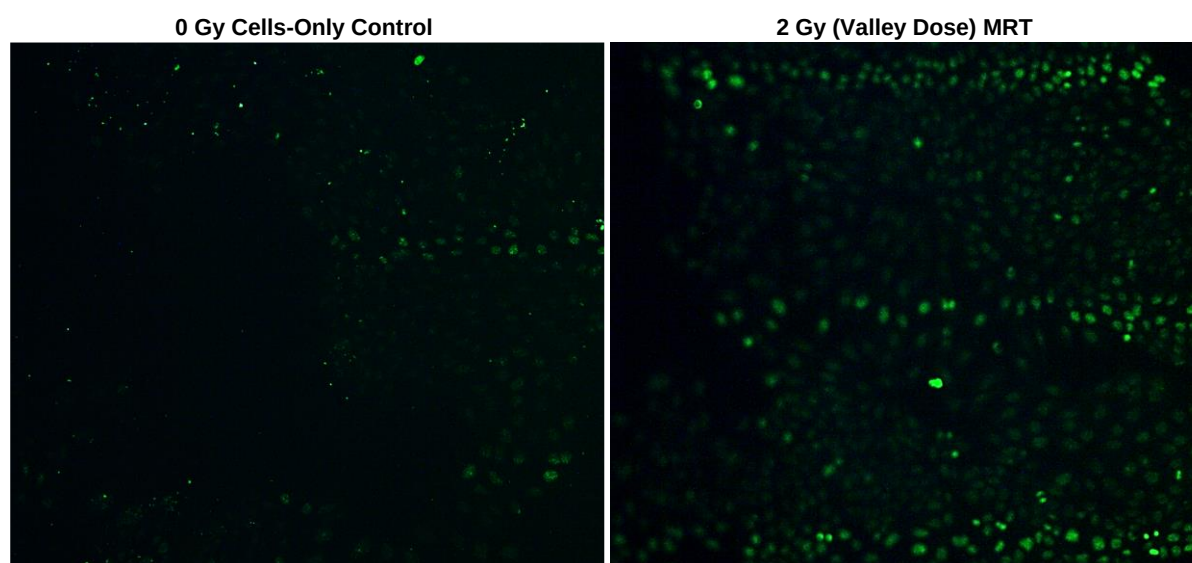
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Supplementary Information

To verify the universality of the FF Model and provide a perspective on its workability with different cell lines, conditions, and more, an additional assessment was performed. Madin Darby Canine Kidney (MDCK) cells, a non-cancerous cell line, was tested using the γ H2AX assay analyzed via the FF method. MDCK cells are a well-established epithelial tissue model suitable for a variety of uses, and provide a widely-used and flexible model for normal, soft tissue assessment.⁵⁸

Fluorescent microscopy was used to acquire the γ H2AX images following method in Fig. 1. A Leica DMi8 fluorescent microscope (Leica Microsystems, Clayton, VIC, AUS) with a 20x dry resolution objective was used with pre-set FITC spectra to capture the γ H2AX foci channel at 488 nm. The images were analysed on the Leica Application Suite X (LASX) software and via ImageJ,⁴⁴ following the analysis method outlined in the FF Model in Fig. 1.

The results of the experiment compared the γ H2AX foci images for MDCK cells irradiated with a 2 Gy (valley dose) MRT field to an untreated (cells-only) MDCK control in Extended Data Fig. 1. A significant sample size of nearly 1000 MDCK cells was imaged and analysed during image processing. A quantification was also performed using the Foci Factor (FF) method to obtain DSBER values, and a BVPDR value. The PVDR value for the 2 T wiggler field and Cu/Al filter (via the same synchrotron facilities described in Tab. 1) used in this irradiation configuration was measured as 8.4, following the method of Engels *et al.*³³



Extended Data Fig. 1 | γ H2AX images of MDCK cells irradiated with MRT. MDCK cells are irradiated with a 2 Gy (valley dose) MRT field with a 2 T wiggler field and a Cu/Al filter (right) and compared with an untreated, cells-only MDCK control (left). The PVDR for this 4-column MRT configuration was 8.4 and mean energy was 71 keV, following the method of Engels *et al.*³³ The green dots present γ H2AX foci, with the fluorescent images acquired at a 20x resolution to capture multiple MRT peaks. All DSBER and BPVDR values quantified using images represented in this figure represent the average of at least three quantified images ($n = 3$) with uncertainty values calculated as standard error of the mean (SEM). Each sample imaged was fixed at 20 min post-irradiation.

Extended Data Fig. 1 was quantified to obtain the DSBER value for the 2 Gy MRT field using Equation 2, with a separate value acquired for peaks and valleys respectively. The peak DSBER was (26.8 ± 6.3) while the valley DSBER was (3.3 ± 0.3). These results are significantly higher than the 0.5 Gy MRT results found for 9LGS and significantly lower than the corresponding 5 Gy MRT 9LGS results (using the same MRT figuration). This is to be expected given a 2 Gy field was used in this case; hence the results fall in the range between the corresponding 9LGS values.

The validation of these results was performed by obtaining a BPVDR value via Equation 3 and comparing to the physical PVDR of 8.4 used for this MRT configuration. The BPDVR value for the MDCK cells was (8.4 ± 2.6), which represents a perfect match. This correlates well with the validation performed in Fig. 5 for 9LGS at multiple MRT doses.

Additionally, several other experimenters verified the results through their own independent trials with the FF model. Experimenters obtained a peak DSBER of (31.3 ± 4.6) and a valley DSBER of (4.3 ± 1.1), while BPVDR = (7.8 ± 2.8). Each of these results were well within the margin of error of the main results for Extended Data Fig. 1, with any differences verified as statistically insignificant ($p \gg 0.05$ for all cases). The universality of the FF method for different experimenters across independent trials was evident.

As such, Extended Data Fig. 1 clearly demonstrated that the FF method can reliably provide highly accurate results for an entirely different cell line, using a differing microscopy technique, and a different radiation dose altogether, even for different experimenters performing independent trials. Of greatest significance, is that the accurate validation of the BPVDR results for MDCK using this method demonstrates the universal reliability of this novel method not just for MRT bio-dosimetry, but for consistent and reliable γ H2AX image quantification more broadly.