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Influence of image acquisition and analysis parameters on γ-H2AX dose-response curves in human lymphocytes exposed to ionizing radiation

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The scoring of γ-H2AX foci as a measure for double-strand breaks (DSB) can be used
to estimate exposure to the very low doses of radiation and shows that the yield of foci induced by ionizing radiation increases linearly with the radiation dose. These findings indicate that quantitation of γ-H2AX foci is a promising tool for biological dosimetry. Quantification of γ-H2AX foci can be achieved by various methods, but the fully automated assessment are a preferred approach for high-throughput biodosimetric analysis. Since the robust calibration dose-response curve is a prerequisite for accurate dose calculation, the factors influencing the measured number of γ-H2AX foci should be known.
The aim of this study was to investigate the influence of various parameters of automated image acquisition and analysis on the slope of γ-H2AX foci dose-response curve.
Peripheral whole blood was irradiated in vitro with 250 kV X-rays (0, 0.2; 0.4; 0.6; 0.8; 1; 1.5; 2; 3; 4 Gy, 1,2 Gy/min), incubated at 37℃ for 30 min and then processed for γ-H2AX foci counting. Images of individual cells were captured and recorded as a training data files with an automated image acquisition and analysis system Metafer (Metasystems, Germany).These files were used to test various parameters dealing with automated cell selection (maximum concavity depth, maximum aspect ratio, minimum and maximum object area), thresholding (object threshold, upper threshold saturation area, minimum upper threshold in % of the gray level range) and spot counting (relative spot area, minimum spot distance, minimum and maximum intensity of a spot). The number of γ-H2AX foci obtained by applying various classifiers was used to construct dose-response curves, which were fitted to a linear model: $y = c + \alpha D$ and compared statistically to each other.
The data obtained revealed that the image acquisition and analysis parameters significantly influence the slope of γ-H2AX foci dose-response curve.

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