

P017 High content analysis of γ H2AX foci by confocal 3D microscopy

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Quantification of DNA damage visualised by identification of γ H2AX foci has the potential to be used to estimate human low-level radiation exposure from clinical therapies or the environment (Rothkamm et al 2007).

Confocal laser scanning microscopy is an extremely versatile tool for biological studies because of the facility to collect 3-dimensional and time resolved data. Currently, quantification of γ H2AX foci is usually carried out manually and this is a tedious and work intensive task. Visual assessment through microscope eyepieces is likely to lead to an underestimate of foci counted because the image examined at any one plane is a 2D image.

Here we describe a procedure for gathering 3D data on γ H2AX foci by confocal laser scanning microscopy and the development of software to facilitate rapid analysis of the foci that provides details of size and location in addition to number.

Custom software was written in MatLab to process and explore the 3D data and detect foci. Foci are located within this initially selected area by the Fernand Meyer watershed algorithm. Once the foci have been marked, statistics are extracted, including centroid position, maximum, minimum and mean intensities within a foci, and equivalent diameters of the foci. Additionally with the centroid data it is possible to study spatial distribution of the foci. The results of this method are dependent on two detection parameters, the initial detection threshold, and the H-maxima threshold.