QUANTIFICATION OF DNA DOUBLE-STRAND BREAKS VIA STORM LOCALIZATION MICROSCOPY

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Double-strand breaks (DSBs) constitute the most dangerous type of DNA damage. By labelling the damage response proteins (γH2AX histone) in the subcellular foci, dSTORM localization microscopy offers a great tool to characterize such lesions. However, the quantitative evaluation of these damage sites poses a challenge, since the number of accepted localizations generated by a single labelled histone (response function) strongly depends on several parameters [1]. The lifetime of the fluorescence ON state ($N_{lifetime}$), the labelling density ($N_{labelling}$), the number of reactivation circles of the applied dye molecules ($N_{activation}$), the localization precision and the length of the linker together determine the size of the blob formed by the accepted localizations of the very same target molecule as you can see in Figure 1. To handle this complex problem, first the response function was statistically given based on a 2D cluster analysis module implemented in the rainSTORM program [2]. Spatial and size distributions of the foci in treated (via NCS and 4-OHT) and untreated U2OS and DIvA cells were quantitatively evaluated. Simulations are also presented to prove the feasibility of the presented 2D evaluations.

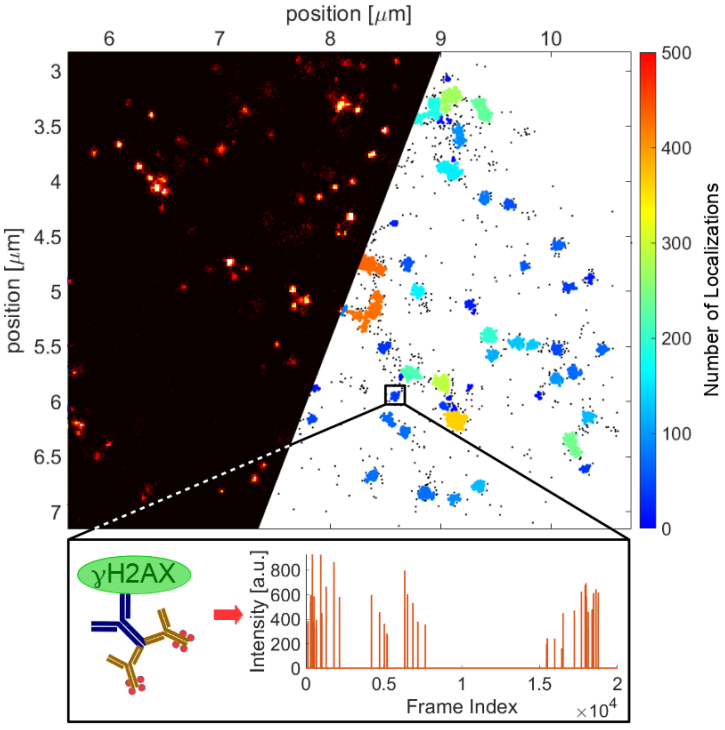

Figure 1: Finding a cluster possibly belonging to an individual labelled histone in a DIvA cell.

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