Quantification of DNA double-strand breaks via dSTORM localization microscopy

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Introduction

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 target molecule (response function) strongly depends on several parameters [1] forming a cluster around the labelled histone.

Results

To handle this complex problem, the response function was statistically given based on a 2D cluster analysis module implemented in the rainSTORM program [2]. Our algorithm allows the quantitative analysis (structure, area and density distributions, etc.) of individual repair focis inside the nucleus of treated (via NCS and 4-OHT) and untreated U2OS and DIvA cells.

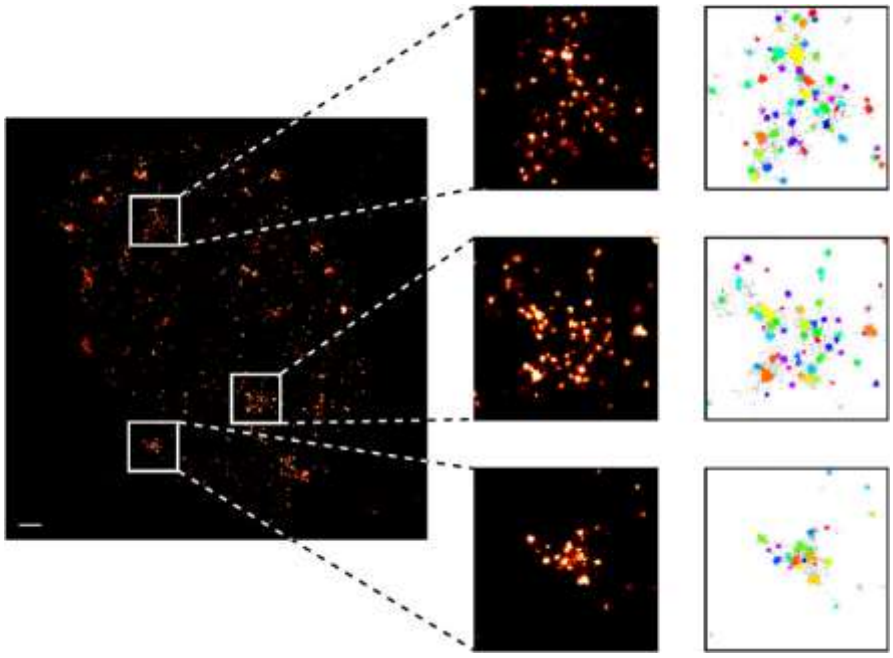

Figure 1. Super-resolved dSTORM image of the entire nuclei of treated DIvA cell. Three typical foci were selected and cluster analysed. Scale bar represents 1 microns.

Conclusion

We could show that dSTORM is the most adequate tool for deep investigation of DNA double-strand break induced repair foci formation, offering new perspectives for further understanding the mechanisms of chromatin function in DNA repair.

References

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