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What can we learn from localization microscopy? New insights into radiation induced changes of the chromatin nanostructure

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Spatial Position Determination Microscopy (SPDM) has become one of the established localisation microscopic techniques that enable effective light optical resolution in the nanometre range even in 3D conserved cell nuclei. It is based on the application of labelling fluorophores that can be switched between two different spectral states (e.g. off/on) to achieve a temporal isolation and thus a spatial separation of the signal molecules. A subsequent computational calculation of these "blinking"events allows the determination of precise positions of the individual labelling molecules as well as the measurement of their spatial distances even if they are considerably below the conventional optical resolution limit. Using SPDM, the nuclear nanostructure and arrangements of euchromatin (EC) and heterochromatin (HC) in irradiated and non-irradiated HeLa cells was investigated after nucleosome labelling via fluorescent proteins and specific antibodies against EC or HC, respectively. In non-irradiated cell nuclei, theoretical approaches of chromatin modelling and statistical analyses revealed a non-random organization of nucleosomes below the 100 nm scale. The sensitivity concerning identification of nanosized structures was proven by changing the labelling site in dimensions of single nucleosomes. Changes of the arrangements of EC and HC regions were measured in terms of distances and molecule densities during repair processes. Besides the nucleosome pattern of H2A and H2B, conformations of EC and HC regions were found to be subjected to different changes due to radiation dose and during repair processes. Image information obtained by SPDM measurements and calculations may offer new insights into the understanding of repair mechanism and support new types of dose-efficiency correlations.

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