Tools for exploring structure and mechanics of chromosomes: Combining adaptive STED nanscopy and optical tweezers

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In our cells, DNA is organized in chromosomes, complex structures of DNA condensed by proteins. To date, it remains a major challenge to visualize and probe the dense and complex molecular architecture of chromosomes. Previously, we combined optical tweezers and one-dimensional stimulated emission depletion (STED) fluorescence microscopy to visualize individual DNA-binding proteins on densely covered DNA and in the presence of high protein concentrations [1]. However, for imaging of more complex and extended three-dimensional (3D) structures such as chromosomes and living cells, control of resolution enhancement in all three spatial dimensions is necessary. Here, we demonstrate a 3D super-resolution imaging system combined with optical tweezers, which is capable of concurrent mechanical manipulation and visualization of chromosomes. A spatial light modulator is used as wavefront controlling element that enables both 3D STED nanoscopy and sensorless adaptive optics [2]. We demonstrate methods to analyze and improve the quality of the engineered point spread function for 2D and 3D STED imaging and present proof-of-principle nanoscopic imaging results. The proposed method can provide superior means to uncover the biological structure and kinetics of vital processes of chromosomes such as their spatial organization during replication.

References:

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