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IT-3-IN-1768 Superresolution Light Microscopy of nuclear Genome Organization

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The spatial organization of the genome in the interphase nucleus has far reaching functional consequences for gene regulation. Recently, various methods of superresolution light microscopy have been developed which made possible to enhance the spatial analysis of nuclear structures far beyond the conventional limits of about 200 nm in the object plane and 600 nm along the optical axis. Here, we report on quantitative nuclear nanostructure analysis based on Structured Excitation Illumination/Structured Illumination Microscopy (SEI/SIM), and on Spectrally Assigned Localization Microscopy (SALM), respectively. Presently, these approaches realized with custom-built systems allow us to optically resolve nuclear structures down to the range of ca. 120 nm laterally/350 nm axially using structured illumination, and few tens of nanometer in 3D using a special variant of localization microscopy, Spectral Precision Distance/Position Determination Microscopy (SPDM). In addition, both SIM and SPDM techniques were combined in a single microscope setup. Application examples will be presented on the use of such ‘nanoscopy‘ approaches to perform quantitative analyses of individual small chromatin domains labelled by Fluorescence-in situ Hybridization (FISH); fluorescence-labelled replication units; of repair foci induced by individual accelerated heavy ions; of Fluorescent-Protein (GFP/YFP/mRFP) tagged histones and chromatin remodeling proteins; or of immunolabelled transcription/splicing related nanostructures. In addition, we report on the direct high resolution SPDM of nuclear DNA distribution, localizing more than 1 million individual DNA sites in an optical section of various types of mammalian cell nuclei. Some perspectives of these novel, quantitative “superresolution” microscopy methods for deciphering the „4D Nucleome“ will be discussed.

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