Nanoscopy of nuclear Genome Structure: Localization and Structured Excitation Illumination Microscopy

C.Cremer¹,²,³* & T. Cremer⁴

¹Institute of Molecular Biology (IMB), D-55128 Mainz/Germany; ²Kirchhoff-Institute of Physics (KIP) and ³Institute for Pharmacy and Molecular Biotechnology (IPMB), University Heidelberg, D-69120 Heidelberg/Germany; ⁴Biozentrum Ludwig-Maximilians-Universität München (LMU), D-82152 Planegg-Martinsried/Germany

*presenting author, e-mail: c.cremer@imb-mainz.de

The spatial organization of the genome in the interphase nucleus has far reaching functional consequences for gene regulation. Both biochemical data and numerical models indicate a decisive role of genome nanostructure; but due to the conventional resolution limits of far-field light microscopy, direct light microscopic tests appeared to be impossible. To overcome these constraints, various methods of superresolution light microscopy/nanoscopy have been developed which made possible to enhance spatial resolution of nuclear structures far beyond the ‘Abbe-limit’. Here, we report on quantitative nuclear nanostructure analysis based on Structured Excitation Illumination Microscopy, and on Spectrally Assigned Localization Microscopy, respectively. Towards this goal, in addition to a commercial OMX microscope we used various custom-built structured illumination and localization microscopes, in combination with a variety of conventional fluorophors and standard preparation conditions. Presently, these approaches allowed us to image fluorescence labeled nuclear structures down to the resolution range of few tens of nanometer. Application examples will be presented for the use of these ‘nanoscopy’ techniques to measure the size of individual small chromatin domains, of replication units, of transcription and repair related structures (collaboration with P. Huber/M. Hausmann, Heidelberg), and to determine the spatial distribution of specific nuclear proteins on the single molecule level. Some perspectives of these novel, quantitative “superresolution” microscopy methods for deciphering the “4D Nucleome” will be discussed.


C. Cremer et al. (2011) Superresolution Imaging of Biological Nanostructures by Spectral Precision Distance Microscopy (SPDM), Biotechnology Journal 6: 1037 – 1051