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Dept. Biology and Biotechnology "Lazzaro Spallanzani"

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SIBBM LECTURE 2020:

Super-Resolution Microscopy of Genome Nanostructure

Prof. Christoph Cremer

Institute for Pharmacy and Molecular Biotechnology (IPMB), University Heidelberg & Kirchhoff-Institute for Physics (KIP); Institute of Molecular Biology (IMB), Mainz (Germany)

Friday 28 February 2020

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Christoph Cremer

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The advent of super-resolution light microscopy (SRM) methods[1,2] has made it possible to elucidate nuclear genome structure[3] on the nanoscale. Here we report on results obtained by Single Molecule Localization Microscopy (SMLM). Using such SRM techniques, the spatial distribution of chromatin in the nucleus and the compaction of individual chromatin domains was measured. The nanoscale DNA distribution across entire nuclei was quantitatively determined by SMLM, applying both photoswitching and spatial switching of standard DNA dyes[4,5]; recently, it became possible to determine in an individual nuclear optical section up to ca. 4 million individual DNA bound single fluorophore molecule positions (ca. 1 position/nucleosome; smallest intranuclear single molecule position distance actually measured ca. 3 nm; best single molecule localization precision ca. 2 nm). Nuclear intensity profile analysis of the intranuclear DNA distributions indicated sharp transitions between high density domains and low density compartments, with differences up to almost two orders of magnitude, while compacted domains had a minimum size down to ca. 30 - 50 nm diameter. In contrast to these results, conventional resolution imaging of the same nuclear sites indicated only small differences in the compaction of different regions, combined with very smooth density transitions across the nucleus. To fully exploit the SMLM to study functional Genome nanostructure inside thick tissues, clearing methods have to be combined with Large Working Distance SRM approaches. As a perspective, the prospects for lens free SRM systems with large working distances (up to the multicentimeter range) are discussed[6].

[1] *C.Cremer, B.R. Masters (2013) Resolution enhancement techniques in microscopy.Eur. Phys. J. H, Eur. Phys. J. H 38: 281–34*

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[3] *T.Cremer et al. (2015) The 4D nucleome: Evidence for a dynamic nuclear landscape based on coaligned active and inactive nuclear compartments. FEBS Letters 589: 2931–2943.*

[4] *I.Kirmes et al. (2015) A transient ischemic environment induces reversible compaction of chromatin. Genome Biology 16:246.*

[5] *A.Szczurek et al. (2017) Imaging chromatin nanostructure with binding-activated localisation microscopy based on DNA structure fluctuations. Nucleic Acids Research. doi: 10.109 3/nar/gkw1301.*

[6] *U. Birk, J. v. Hase, C. Cremer (2017) Super-resolution microscopy with very large working distance by means of distributed aperture illumination. Sci. Rep. 7: 3685; | doi:10.1038/s41598-017-03743-4 1.*

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