

Quantitative Super Resolution Microscopy of DNA compaction in Mammalian Cell Nuclei

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Abstract

Super-resolution light microscopy (SRM) methods have provided new possibilities to investigate quantitatively the spatial consequences of current models of nuclear genome organization at nanoscale resolution.¹ We have proposed a model, where higher order chromatin networks are built up from chromatin nanodomains and embedded in a low DNA density environment, called the interchromatin compartment (IC). The IC is lined by chromatin enriched with RNA Pol II and histone markers indicating transcriptional competence.^{2,3} Numerical simulations indicate that the accessibility of chromatin nanodomains for macromolecule complexes may be substantially restricted at DNA densities $\geq 50 \text{ Mbp}/\mu\text{m}^3$ and thus play a major role in transcriptional control⁴. Here, we describe ongoing studies to measure the absolute compaction of chromatin nanodomains, including individual genes, which were recorded with Structured Illumination (SI) and Single Molecule Localization Microscopy (SMLM). Using SI, the DNA compaction of specifically labelled gene domains yielded DNA density estimates with peak values $>50 \text{ Mbp}/\mu\text{m}^3$. SMLM has been applied to measure the overall DNA density distribution across entire nuclei. An individual nuclear optical section carries up to ca. 4 million individual positions of DNA-bound, single fluorophore molecules (ca. 1 position/nucleosome) and revealed compacted domains with a minimum size down to ca. 30 - 60 nm diameter. The average lateral resolution in this 2D section was about 40 nm, the axial resolution about 600 nm. Intensity profile analyses of the intranuclear DNA distributions indicate sharp transitions with differences of nearly two orders of magnitude between chromatin nanodomains with high and low DNA densities. Instrumentation for nuclear sectioning with increased axial resolution (3D SMLM) has been developed and will be used together with a Voronoi tessellation approach (collaboration O. Kröger/F. Sadlo, Univ. Heidelberg) for measurements of absolute DNA densities ($\text{Mbp}/\mu\text{m}^3$) of chromatin nanodomains located either in the active nuclear compartment (ANC) or in the co-aligned inactive nuclear compartment (INC).²

¹C. Cremer et al. (2017) *Super-resolution microscopy approaches to nuclear nanostructure imaging. Methods* **123**:11- 32; ²T. Cremer et al. (2020) *The Interchromatin compartment participates in the structural and functional organization of the cell nucleus. BioEssays* **42**: 1900132; ³I.Kirmes et al. (2015) *A transient ischemic environment induces reversible compaction of chromatin. Genome Biology* **16**:246. ⁴K. Maeshima et al. (2015). *The physical size of transcription factors is key to transcriptional regulation in chromatin domains. J Phys Condens Matter* **27**, 06411.