Voronoi-Tessellation based High Content Nanoscopy of nuclear Genome Structure

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Abstract

Super-resolution light microscopy (SRM) methods[1] have provided new possibilities to investigate quantitatively the spatial consequences of current models of nuclear genome organization[2] at nanoscale resolution. To achieve the high structural resolution required, "high content" approaches of Single Molecule Localization Microscopy (SMLM) have been developed, such as fluctuation based binding activated localization microscopy (fBALM)[3,4]. Using this technique, up to about 10 million individual DNA sites in an optical section of an individual mammalian cell nucleus have been localized with high precision, corresponding to about 1 Single Molecule localization (SM) per 60 base pairs, or about 3 per nucleosome. To draw from such large amounts of SM positions meaningful conclusions with respect to nuclear chromatin organization, a variety of algorithms may be used. Here, we report on recent results of a Voronoi tessellation approach applied to the entire data set. This allowed us to determine the absolute 3D DNA densities of chromatin assemblies in entire nuclear sections at a lateral resolution of 10-20 nm, and an axial resolution down to 600 nm. The results revealed a high heterogeneity of the DNA density distribution, with absolute 3D DNA compaction levels ranging from a few Mbp/µm^3 up to >40 Mbp/µm^3. Our results support a model[2], where higher order chromatin networks are built up from chromatin nanodomains, pervaded by a contiguous network of interchromatin channels, called the interchromatin compartment (IC). How this structure of the nuclear landscape and its space-time dynamics may affect the accessibility of nanodomains for individual factors and pre-assembled aggregates remains to be explored.

*[1]C. Cremer et al. (2017) Super-resolution microscopy approaches to nuclear nanostructure imaging. Methods **123**:11- 32; [2]T. Cremer et al. (2020) The Interchromatin compartment participates in the structural and functional organization of the cell nucleus. BioEssays **42**: 1900132; [3]A.Szczurek et al.(2017) Imaging chromatin nanostructure with binding-activated localisation microscopy based on DNA structure fluctuations. Nucleic Acids Research. 45(8):e56. [4]A.Szczurek et al. (2018) Super-Resolution Binding Activated LocalizationMicroscopy through reversible change of DNA conformation. Nucleus **9**: 182 – 189.*