INDUCING DNA STRUCTURE FLUCTUATIONS AS A TOOL FOR IMAGING CHROMATIN NANOSTRUCTURE WITH BINDING-ACTIVATED LOCALISATION MICROSCOPY (fBALM)

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KEY WORDS: fBALM, SMLM, chromatin, nuclear nanostructure, DNA binding dyes.

Emerging methods of super-resolution light microscopy combined with novel developments in fluorophore chemistry and labeling techniques provide the basis for nanostructure analysis of chromatin [1,2,3]. We have developed a general scheme for Single Molecule Localization Microscopy (SMLM) imaging of DNA-binding dyes, which is based on conjointly modifying the properties of DNA, the dye, and the imaging media [4]. A careful adjustment to the chemical environment and the control of the time-dependent changes in the imaging buffer can be utilized to induce a local, reversible DNA melting and re-annealing. These structure fluctuations can be used as an alternative method to gain control over the fluorescence signal of DNA-binding dye molecules, as a variation of binding-activated localization microscopy (BALM) [1], which was termed fBALM [4]. The approach can be optimized in order to register only a few optically isolated DNA-binding dye signals at a time (i.e. in each frame). The method has been verified using a number of DNA binding dyes from both DNA intercalators and minor-groove binders. With these developments we were able to characterize differences in nuclear architecture on the nanoscale. As an example application, we captured images of intact nuclei after induction of model ischemia; indicating a structural resolution of approximately 50 nm. Potential applications of this SMLM analysis of chromatin nanoarchitecture include, for instance, the microscopic study of nuclear structure aberrations and their relation to various diseases. The structural resolution obtained using our novel approach in principle permits the study of nuclear nanostructure differences correlated, e.g., to different stages of development, or to environmental stress conditions.

REFERENCES

[1] I. Schoen et al. *Nano Letters*, doi: 10.1021/nl2025954 (2011).
[2] I.Kirmes et al. *Genome Biology*, doi:10.1186/s13059-015-0802-2 (2015).
[3] T. Cremer et al. *FEBS Letters* 589: 2931–2943(2015).
[4] A. Szczurek et al. *Nucleic Acids Research* doi:10.1093/nar/gkw1301 (2017).