SMLM-ConText, A SOFTWARE TOOL FOR THE ANALYSIS OF CONFORMATION AND TEXTURE IN SINGLE MOLECULE LOCALIZTION MICROSCOPY IMAGES

Amine Gourram[1,2], Aleksander Szczurek[1], Jan Neumann[3], Christoph Cremer[1,2,4], and Udo J. Birk[1,2]

[1]Institute of Molecular Biology (IMB), Mainz, Germany; [2]University Mainz, Institute of Physics, Germany; Max-Planck Institute for Chemistry (MPIC), Mainz, Germany; [4]University Heidelberg, Kirchhoff Institute for Physics (KIP), Heidelberg, Germany

E-mail : u.birk@imb-mainz.de

KEY WORDS: SMLM, conformation, texture, chromatin, nuclear nanostructure.

The image quality obtained in Single Molecule Localization Microscopy (SMLM) experiments in principle permits representation of biological structures with a resolution in the 10 nm range. However, analysis of the texture and conformation of the structures observed in these images is hampered by the lack of suitable image analysis tools. For instance, nuclear chromatin during interphase forms structures at the nanoscale, with distinct functional units of various degree of condensation [1]. The interdependency of arrangement, condensation and level of activity is subject of ongoing research. Chromatin nano-architecture cannot easily be observed with conventional microscopy due to the limited optical resolution. To overcome this problem, we have recently developed methods for super-resolution imaging of nuclear DNA using single molecule localization microscopy (SMLM) which are able to provide images of chromatin nanostructures down to sizes of about 40 nm [2,3].

Here, we introduce **SMLM-ConText** (conformation and texture), a new software tool for the quantitative analysis of the distribution of signals from DNA-binding dye molecules in SMLM images. **SMLM-ConText** uses a combination of pixel size optimization, thresholding, morphological operations, and edge detection to yield a single conformation and texture parameter (CTP) describing e.g. the nuclear chromatin organization on the single cell level, allowing us to quantify and differentiate between nuclei with low, medium, and high level of chromatin condensation.

We applied this software to experimental SMLM data obtained from two sample populations of mouse cardiomyocyte HL-1 cells, one population subjected to oxygen and nutrient deprivation (OND), and a control group. We found that the CTP decreases drastically upon OND treatment and hence is correlated to gene activity. We applied the method to a third population of cells (neuroblastoma, Neuro2a), and compared the results to those obtained from the HL-1 cells. The differences for the CTP were found to be statistically highly significant.

Our software is robust, fast, and easy to use as it requires only two input parameters. It promises a valuable tool for routine application of SMLM microscopy (e.g. in clinical diagnostics) not only for analysis of nuclear chromatin on the single cell level, but also for applications on both cultured cells and tissue sections, e.g. related to neurodegenerative diseases, cell differentiation, cancer treatment and drug development.

REFERENCES

[1] T. Cremer et al. *FEBS Letters*, doi:10.1016/j.febslet.2015.05.037 (2015).
[2] I.Kirmes et al. *Genome Biology*, doi:10.1186/s13059-015-0802-2 (2015).
[3] A. Szczurek et al. *Nucleic Acids Research*, doi:10.1093/nar/gkw1301 (2017).