ADVANCED fBALM IMAGING OF NUCLEAR NANOSTRUCTURES BY AUTOMATED BUFFER EXCHANGE

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Single molecule localization microscopy (SMLM) circumvents the diffraction limit of conventional light microscopy by imaging subsets of optically isolated single fluorescent molecules (for a review see [1]). The position of a single isolated molecule can be determined with a precision below the optical diffraction limit. The required temporal and spatial optical isolation is achieved by switching between spectral on and off states [2]. Combining all detected positions to a joint localization map finally results in an image with enhanced resolution. However, the application of SMLM methods in long-term experiments is challenging.

Here we present an approach for long-term measurements of advanced DNA fluctuation assisted Binding Activated Localization Microscopy (fBALM) by using a micro pump system [3]. fBALM relies on the spatial switching of fluorescent on/off states by adjusting the local environment of the DNA and DNA-binding dyes. The chemical environment allows control over the local, reversible DNA melting and hybridization and can be used to induce optical isolation by transient binding of the dye.

fBALM methods so far employ the glucose oxidase (GOX) and catalase (CAT) enzymatic oxygen scavenging system to gradually change the pH of the imaging buffer over time and thereby preserve the nanostructure of the cell nucleus [4]. The use of a micro pump system now allows the controlled adjustment of the pH-value of the buffer without employing the GOX/CAT oxygen scavenging system.

We evaluate the optimal pH-value for fBALM measurements, and investigate the influence of the pump system on the imaging quality. We show fBALM measurements of a cell nucleus after gradually decreasing the pH of the imaging buffer and highlight the possibility of relabeling after several hours at low pH values on the sample, recovering the initial fBALM image quality. We can relocate cells within a precision of 10 nm on the sample. This opens the perspective for long-exposure and multicolor super-resolution microscopy imaging of the cell nucleus.

REFERENCES

[1] C. Cremer, and B.R. Masters “Resolution enhancement techniques in microscopy”, *The European Physical Journal H*, **38**, 281-344, (2013).

[2] P. Lemmer et al. “SPDM: light microscopy with single-molecule resolution at the nanoscale”, *Applied Physics B*, **93(1)**, 1-12 (2008)

[3] P. Almada et al. “Automating multimodal microscopy with NanoJ-Fluidics”, *bioRxiv* 320416 (2018)

[4] A. Szczurek et al. “Imaging chromatin nanostructure with binding-activated localization microscopy based on DNA structure fluctuations” *Nucleic Acids Res.*, **45**, e56 (2017).