Sample drift estimation method based on speckle patterns formed by backscattered laser light

Shih-Ya Chen[1], Rainer Heintzmann[2,3], Christoph Cremer[1,4,5*]

[1]Institute of Molecular Biology, Mainz, Germany

[2]Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller-University Jena, Jena, Germany

[3]Leibniz Institute of Photonic Technology, Jena, Germany

[4]Department of Physics, University of Mainz (JGU), Mainz, Germany

[5]Institute for Pharmacy and Molecular Biotechnology (IPMB), and Kirchhoff Institute for Physics (KIP), Heidelberg, Germany

*c.cremer@imb-mainz.de;cremer@kip.uni-heidelberg.de

Abstract

Single molecule localization microscopy (SMLM) has been established to acquire images with unprecedented resolution down to several nanometer. A typical time scale for image acquisition is several minutes to hours. Yet it is difficult to avoid completely sample drift for long time measurements, with the consequence of deterioration of resolution.

To correct sample drift, we present a method based on the evaluation of speckle patterns formed by laser light back-scattered from cells using a single molecule localization microscope setup [1]. A z-stack of unique speckle patterns is recorded prior the measurements as a three dimensional position reference. During the experiment, images of scattered laser light were acquired. Afterwards an image registration method based on nonlinear optimization and discrete Fourier transforms (DFTs) was used to correlate the scattered laser images individually with each of the images from the speckle reference stack [2]. From this, the drift information in x, y and z was acquired and used for positional correction down to the < 10 nm range

Our method shows highly comparable results with a fiducial marker approach, achieving a precision of several nanometer. This method can also benefit for the three dimensional stabilization of microscopy systems without any additional sample preparation.

We demonstrated this method for imaging chromatin nanostructure in cell nuclei in 3D. Based on the fBALM [3] method, a SMLM experiment was performed. Several small chromatin clusters were found in the super-resolution image of the nucleus. After drift correction, more confined nucleosome domain clusters in 3D were revealed.

[1] S.-Y. Chen, R. Heintzmann, and C. Cremer, "Sample drift estimation method based on speckle patterns formed by backscattered laser light," *Biomed. Opt. Express*, 10, 6462-6475 (2019).

[2] M. Guizar-Sicairos, S. T. Thurman, and J. R. Fienup, “Efficient subpixel image registration algorithms,” *Opt. Lett.*, 33, 156–158 (2008).

[3] A.Szczurek, L. Klewes, J. Xing, A. Gourram, U. Birk, H. Knecht, J. W. Dobrucki, S. Mai, C. Cremer, “Imaging chromatin nanostructure with binding-activated localisation microscopy based on DNA structure fluctuations,” *Nucl. Acids Res*.,45,e56 (2017).