Patterned illumination single molecule localization microscopy (piSMLM): user defined blinking regions of interest

Shih-Ya Chen[1], Felix Bestvater[2], Wladimir Schaufler[2,5], Marton Gelleri[1], Rainer Heintzmann[3,4], Christoph Cremer[1,5]

[1]Institute of Molecular Biology, Ackermannweg 4, D-55128 Mainz, Germany

[2]German Cancer Research Center, D- 69120 Heidelberg, Germany

[3]Institute of Physical Chemistry and Abbe Center of Photonics

D-07743 Jena & [4]Leibniz Institute of Photonic Technology, D-07745 Jena, Germany

[5]Institute of Pharmacy and Molecular Biotechnology, D-69120 Heidelberg, Germany

Email: c.cremer@imb-mainz.de

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We present a newly developed patterned illumination single molecule localization microscope (piSMLM) to overcome the problem of noneven illumination and which offers the possibility to define arbitrarily shaped illumination patterns by computer-generated holography (CGH).

By utilizing a phase only spatial light modulator (SLM) in combination with a modified Gerchberg-Saxton algorithm, a user-defined pattern with homogeneous illumination can be obtained. Our experimental results show that an illumination intensity of 1 to 5 kW/cm^2 was achieved by using a laser with an output power of 200 mW. Higher intensities up to 20 kW/cm^2 can be reached by simply reducing the size of the region of interest. To demonstrate the capability of piSMLM, a cell nucleus as an arbitrary shape was selected for the patterned illumination between two daughter cells.

The pattern illumination method is not only restricted to the application for SMLM. The flat-top illumination can also be applied to high-throughput microscopy by generating e.g. a square shaped illumination area. Other applications such as optogenetic or fluorescence recovery after photobleaching (FRAP) will also benefit from the freedom of defining single or multiple ROIs for light stimulation.

Reference:

1. R. W. Gerchberg and W. O. Saxton, “A practical algorithm for the determination of phase from image and diffraction plane pictures” Optik 35(2), 237-250 (1972).

2. Shih-Ya Chen, Felix Bestvater, Wladimir Schaufler, Rainer Heintzmann, and Christoph Cremer, "Patterned illumination single molecule localization microscopy (piSMLM): user defined blinking regions of interest," Opt. Express 26, 30009-30020 (2018).