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

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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/cm² was achieved by using a laser with an output power of 200 mW. Higher intensities up to 20 kW/cm² 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: