HOMOGENEOUS MULTIFOCAL EXCITATION FOR HIGH-THROUGHPUT SUPER-RESOLUTION IMAGING

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Super-resolution microscopies, which allow features below the diffraction limit to be resolved, have become an established tool in biological research. However, imaging throughput remains a major bottleneck in using them for quantitative biology, which requires large datasets to overcome the noise of the imaging itself and to capture the variability inherent to biological processes.

Here, we develop a multi-focal flat illumination for field independent imaging (mfFIFI) module, and integrate it into an instant structured illumination microscope (iSIM)[1]. Our instrument extends the field of view (FOV) to >100x100 μm^2 without compromising image quality, and maintains high-speed (100 Hz), multi-color, volumetric imaging at double the diffraction-limited resolution. We further extend the effective FOV by stitching multiple adjacent images together to perform fast live-cell super-resolution imaging of dozens of cells. Finally, we combine our flat-fielded iSIM setup with ultrastructure expansion microscopy (U-ExM)[2] to collect 3D images of hundreds of centrioles in human cells, as well as of thousands of purified *Chlamydomonas reinhardtii* centrioles per hour at an effective resolution of ~35 nm. We apply classification and particle averaging to these large datasets, allowing us to map the 3D organization of post-translational modifications of centriolar microtubules, revealing differences in their coverage and positioning.

This novel high-throughput approach lends possibility to combining super-resolution with more advanced kinds of analysis, such as high-content screening, structural modelling and machine learning applications.

References:

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