MARRYING SOFI WITH SIM FOR LARGE FIELDS-OF-VIEW SUPER-RESOLUTION IMAGING

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Abstract

All fluorescence super-resolution microscopy techniques present trade-offs, for example between resolution, acquisition speed and live-cell compatibility. Structured Illumination Microscopy (SIM) improves the resolution through successive imaging of the sample under patterned illumination. SIM can be fast and typically uses low light levels well suited for live cell imaging. However, in its linear form, the resolution is restricted to a twofold improvement over the diffraction limit. Super-resolution optical fluctuation microscopy (SOFI) is an alternative to localization microscopy that is less demanding in terms of fluorophore photoswitching, offering better time resolution and lower phototoxicity at the cost of a more moderate resolution gain. SOFI analyzes spatio-temporal fluctuations in fluorescence by calculating higher-order cumulants, a quantity related to correlations. The resolution improvement scales with the cumulant order n. In practice, the resolution gain is limited by the signal-to-noise ratio and the need for an increased number of frames. Here, we demonstrate the combination of SOFI with SIM where we use SOFI as a source of non-linearity to further enhance the SIM resolution.

We will present two implementations of SIM combined with self-blinking dyes for SOFI. The first uses a new Michelson SIM setup for achromatic high-efficiency illumination and fast pattern projection. We acquired single and two-color SIM data of blinking emitters with up to 2.4 fold image resolution increase. We applied the same concept to realize SOFI-SIM on a flat-fielded, high-throughput instant SIM (iSIM) setup, achieving similar resolution and demonstrating the versatility of our approach. We will also discuss the SOFI-SIM reconstruction challenges and emphasize how spontaneously blinking fluorophores facilitate the experiments.

The combination of the two methods reaches a resolution beyond what is achievable with SIM alone without increased complexity of the experiment. We consider the presented implementations a promising alternative for enhancing the resolution in biological imaging at low illumination levels with reasonable acquisition speed.