Super-Resolved Total Internal Reflection Fluorescence Microscopy using Random Illuminations, TIRFRIM

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Abstract

Random Illumination Microscopy (RIM) is a recent super-resolution fluorescence microscopy technique in which the sample is illuminated by random speckled patterns. The reconstruction is formed from the multiple (from 50 to several hundreds) speckled images using a variance matching inversion scheme accounting for the auto-correlation of the speckles. The strength of (RIM) is to offer the resolution gain and linearity to brightness of Structured Illumination Microscopy (SIM) using a simple experimental set-up with minimally controlled illuminations [1]. Here, we implemented RIM in the Total Internal Reflection Fluorescence configuration for imaging biological processes close to the coverslip surface [2]. We generated the evanescent speckled illumination by placing a ring mask at the Fourier plane of the microscope objective and a diffuser at its image plane. Thanks to its quasi insensitivity to aberrations on the illumination side, TIRF-RIM was shown to be significantly less affected by artefacts than TIRF-SIM [2]. Using standard TIRF-objectives, TIRF-RIM was able to separate fluorescent lines 60 nm apart and to distinguish the ring-shape of the clathrin coated pits (Fig. 1). Applied to live macrophages, TIRF-RIM provided two-color dynamic images of paxillin nanoclusters with spatial (120 to 96 nm) and temporal (8 to 1 Hz) resolutions. While TIRF-SIM is adapted to situations where very high temporal resolution and low photon budget are required, we believe that TIRF-RIM robustness and simple experimental set-up make this novel approach a method of choice for high throughput screening at super-resolution.

References

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