Medium Content FAST 3D RIM Superresolution Microscopy for live Biological Imaging : from cell culture to tissues.

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Abstract

We describe here a new version of RIM (random illumination microscopy (RIM), called FAST RIM, for live-cell imaging at super-resolution matching best 3D SIM and with unprecedented optical sectionning. The principle of RIM consists in studying the statistics of images recorded for different realizations of speckles. It replaces the knowledge of the illuminations, necessary in the deterministic carrier techniques, by the knowledge of the statistics of the illuminations. The super-resolved reconstruction is obtained numerically, from the variance of the speckled images and the autocorrelation function of speckles, using a variance matching algorithm, named AlgoRIM [1-2-3]. RIM is unaffected by optical aberrations on the excitation side, linear to brightness, and compatible with multicolor live-cell imaging over extended periods of time [1]. RIM requires the acquisition of a hundred of images under various illuminations of the sample. In an ideal case where the acquired images are not noisy, there remains a residual fluctuation in the variance image related to the limited number of acquisitions. Unfortunately for constraints related to the imaging of living organisms, it is often not possible to work with a large number of images. For a limited number of acquisitions ~50, these fluctuations can be significant and even greater than that related to the measurement noise. It is possible to generate a set of illuminations to minimize these fluctuations by optimizing pattern using a low cost binary SLM to reduce the effect of these fluctuations. We have developed a medium content versions of RIM, called FAST RIM, on a basic microscope that allows the recording in two colors of 1300 images per second, using a synchronization between the illumination and the CMOS detector significantly simpler than that used in SIM technology. Optimized speckle (between 12 to 48) increased the temporal resolution and the medium content screening of 3D RIM applications. New algorithm based on the standard variation allows real time reconstruction during acquisition in wide field of view (110µmx110µm). The methods increase 3D imaging and the reproducibility medium content imaging in optical aberrant conditions like expansion microscopy [4], or in live imaging of focal adhesions from human osteoclasts on bone [5]. Finally, the increase of robustness reveals for the first time novel chromatin compartment that regulates the response to DNA Double-Strand Breaks and the biogenesis of translocations [Arnoud, in rev]. Axial resolution about 200 nm and transverse resolution of about 120 nm have been obtained tens of microns deep inside biological tissues at 8 HZ temporal resolution, in conditions difficult for SIM (large Stoke shifts and strongly aberrating medium).

References

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