Three-dimensional fluctuation-based super-resolution bioimaging

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Record-holding super-resolution microscopy (SRM) techniques, e.g. localization microscopy (LM) and stimulated-emission-depletion (STED) microscopy, require significant resources and effort from life-science researchers. In comparison with confocal microscopy, apart from relying on extensive sample-staining procedures and long acquisition times, applying LM for 3D and multicolor imaging poses a considerable experimental challenge. We provide a complete demonstration of an entry-level SRM technique – providing super-resolving capabilities with an experimental complexity level akin to that of confocal microscopy [1]. Exchanging the confocal pinhole with a small pixelated detector, we use the inherent fluctuations of dye molecules as contrast for SRM. This contrast is processed into a super-resolved image through a process of pixel reassignment, a robust and deterministic algorithm. Since the fluctuation contrast is ubiquitous to organic markers, it does not require engineering of the blinking statistics through the sample buffer. Together with the built-in capabilities for multi-color and 3D imaging, shown in our work [1], it can become a natural extension to confocal microscopy - a straightforward first step into the realm of SRM.

In our implementation we rely on a single photon avalanche diode (SPAD) array [2]. Its spatial resolution enables resolution enhancement through Image scanning microscopy (ISM [3]). The temporal resolution offers further improvements through the measurement of quantum [4] or classical [5] correlations in the emitted fluorescence, also extending the applications to fluorescence lifetime imaging (FLIM, [6]) and other methods based on time correlated single photon counting (TCSPC). TCSPC often requires significant measurement times, compromising spatial overlap of the color channels when sequential measurements are performed. Taking advantage of the intrinsic temporal resolution of SPADs, we utilize pulsed interleaved excitation to temporally multiplex fluorescence color channels, alleviating the need to measure one color at a time, and keeping the experimental setup simple. This enables multicolor TCSPC measurements yielding FLIM and correlation-based super-resolution, without the need for channel alignment in post processing — each position in the sample is measured simultaneously in available channels.

In the second experiment [7], we combine Super-resolution Optical Fluctuation Imaging (SOFI) [8] with temporal focusing two-photon excitation [9] – a wide-field microscopy method that is capable of excitation of a thin slice in a three-dimensional specimen. Both methods are straightforward to implement in a standard microscope, and by merging them, we obtain super-resolved 3D images of neurons stained with quantum dots. Our approach offers reduced bleaching of out-of-focus planes and an improved signal-to-background ratio that can be used when robust resolution improvement is required in thick, dense samples.

Acknowledgements

This work was supported by the Foundation for Polish Science under the FIRST TEAM project “Spatiotemporal photon correlation measurements for quantum metrology and super-resolution microscopy” co-financed by the European Union under the European Regional Development Fund (POIR.04.04.00-00-3004/17-00), by the National Science Centre, Poland, grant number 2022/47/B/ST7/03465, and Horizon Europe MSCA FLORIN project ID 101086142

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