SUPER-RESOLVED CONFOCAL FLUCTUATION MICROSCOPY USING A SPAD ARRAY

Adrian Makowski[1], Ron Tenne[2,3], Gur Lubin[3], Aleksandra Sroda[1], Michel Antolovic[4,5], Uri Rossman[3], Edoardo Charbon[4], Claudio Bruschini[4], Dan Oron[3] and Radek Lapkiewicz[1]

[1]Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Poland
[2]Department of Physics and Center for Applied Photonics, University of Konstanz, Germany
[3]Department of Physics of Complex Systems, Weizmann Institute of Science, Israel
[4]School of Engineering, Ecole polytechnique fédérale de Lausanne (EPFL), Neuchâtel, Switzerland
[5]Pi Imaging Technology SA, Neuchâtel, Switzerland
Email: radek.lapkiewicz@fuw.edu.pl, a.makowski2@student.uw.edu.pl

KEY WORDS: SOFI, ISM, SPAD array

Super-resolution techniques, such as PALM, STORM, and STED, offer excellent spatial resolution in the range of tens of nanometers. PALM and STORM rely on spectroscopic properties of fluorophores used to stain the samples and long data acquisition, whereas STED requires a high power laser and a complex experimental setup. Alternative methods breaking the diffraction limit, such as image scanning microscopy (ISM) [1,2] and super-resolution optical fluctuation imaging (SOFI) [3], are relatively straightforward to implement but produce images with a modest resolution improvement. ISM requires replacing a single detector in a confocal microscope with a detector array, whereas SOFI relies on the temporal analysis of light intensity emitted by independently fluctuating fluorescence emitters.

Here, we enhance the resolution of ISM by performing a fluctuation correlation analysis in an ISM architecture. Our technique, which we termed *super-resolution image scanning microscopy* (SOFISM)[4], enables obtaining images with resolution higher than the one produced by either method alone (e.g. a lateral resolution enhancement up to 4 times beyond the diffraction limit for second-order correlations). Obtaining such an image requires capturing fast, sub-millisecond, intensity fluctuations. To do so we employ a high-performance 23-pixel SPAD array [5,6]. The use of a fast and sensitive, all-solid-state array of single-photon detectors in SOFISM facilitates reasonable correlation measurements within a few milliseconds exposure per pixel, much faster than compared with at least a few seconds required for a standard camera, which averages out high-frequency fluctuations of fluorophores.

In summary, we demonstrate that equipping a confocal microscope with a SPAD array detector enables achieving a resolution enhancement in all three dimensions within standard acquisition times.

[1] C. B. Muller, J. Enderlein, “Image scanning microscopy” *Phys. Rev. Lett*. **104**, 198101 (2010)
[2] C. J. R. Sheppard, ”Superresolution in confocal imaging,” *Optik* **80**, 5354 (1988)
[3] T. Dertinger, R. Colyer, G. Iyer, S. Weiss, and J. Enderlein, “Fast, background-free, 3D super-resolution optical fluctuation imaging (SOFI),” *Proc. Natl. Acad. Sci.* **106**, 22287–22292 (2009)
[4] A. Sroda, A. Makowski, R. Tenne, U. Rossman, G. Lubin, D. Oron, and R. Lapkiewicz, “SOFISM: Superresolution optical fluctuation image scanning microscopy,” *Optica* **7**, 1308 (2020)
[5] I. M. Antolovic, C. Bruschini, and E. Charbon, ”Dynamic range extension for photon counting arrays” *Opt. Express* **26**, 22234- 22248 (2018)
[6] SPAD23system.pdf (piimaging.com)