IMAGE SCANNING MICROSCOPY DIVES DEEP, WITH SPAD ARRAY AND BLIND IMAGE RECONSTRUCTION

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Diffraction-unlimited optical nanoscopy techniques today can reach resolution down-to only a few nanometers, but they have not been able to replace traditional fluorescence, confocal and two-photon microscopes as go-to imaging tools in pre-clinical research. The old techniques are reliable, simple, familiar, highly compatible with all kinds of fluorescence labels and work well with many types of samples, whereas current nanoscopy techniques fall short on at least some of these characteristics.

Two-photon microscopy is the imaging modality of choice, when one desires to observe thick, biological samples, possibly in-vivo. However, the resolution in two-photon microscopy is poor, and the lack of an optical pinhole quickly becomes apparent in complex samples as reduced quality of optical sectioning. Here we propose a straightforward implementation of two-photon image scanning microscopy (2PE-ISM) that, by leveraging our recently introduced ISM platform [1] – based on a new single-photon avalanche diode (SPAD) array detector – is shown to improve both the optical resolution and optical sectioning in various test samples.

We also discuss new image reconstruction aspects that the new ISM platform enables. It is for example possible to detect and compensate for optical aberrations at the post-processing stage. Our new blind adaptive (multi-image) deconvolution algorithm [2] is shown to produce ISM results with unprecedented contrast and effective resolution. Most importantly our (2PE-)ISM image reconstruction requires no input from the user: the optimal image reconstruction parameters and/or the point-spread-function are directly estimated from the data. As a result, our 2PE-ISM system works like any old and familiar two-photon system, but simply produces higher resolution images. Making the complexity disappear, in our view, is the biggest novelty here, and the key for making super-resolution deep imaging truly feasible.

References

[1] Castello, M. *et al.* A robust and versatile platform for image scanning microscopy enabling super-resolution FLIM. *Nat. Methods* (2019). doi:10.1038/s41592-018-0291-9
[2] Koho S, Tortarolo G, Castello M, Deguchi T, Diaspro A, Vicidomini G. Fourier Ring/Shell Correlation Simplifies Image Restoration in Fluorescence Microscopy. *in-review*