COMPUTATIONAL METHODS LEVERAGING SPAD ARRAY ENABLE DEEP SUPER-RESOLUTION IMAGING IN LASER-SCANNING MICROSCOPES

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Super-resolution techniques, introduced in the early 2000s have given birth to new interest in optical (fluorescence) microscopy research. A plethora of new microscopy techniques have been proposed, some able to reach molecular scale resolution and others able to image large samples, even whole embryos, at unprecedented speeds. A second revolution in the microscopy research community is currently ongoing with the integration of advanced computational methods, both analytical and machine learning based, to microscopy techniques [1]. The new computational methods are enabling new kinds of microscopy techniques, they can dramatically boost the quality of image data that just recently would have been thrown away as garbage [2] and they are also enabling a comeback of sorts for traditional imaging methods, such as confocal and two-photon (2PE) microscopy that continue to be the favorite imaging tools in the research community. We recently introduced a new asynchronous readout SPAD array detector [3], which in combination with computational image reconstruction, improves the resolution in confocal microscopy by a factor of two, while also significantly boosting the signal-to-noise ratio. Recently we have integrated the same detector to a two-photon microscope, with even more dramatic results [4]. This is partially thanks to a new set of semi-blind image reconstruction and restoration methods that were tightly integrated into the microscope [5]. A second, and possibly more ground-breaking benefit of adding an array detector in a point scanning microscope is that the data collected by the detector also contains information of optical aberrations. Here we discuss, how this information can be used to further improve the performance of the microscope. We show how our adaptive pixel reassignment image reconstruction algorithm can both reveal and correct optical aberrations. We also discuss that it is possible to obtain quantitative measures of those aberrations computationally. No separate wavefront sensor or sequential image-based optimization is required. As we see it, this discovery will open up a way for much simplified adaptive optics systems, which even a regular microscope user might be able to use. This may have dramatic implications for enabling easy deep super-resolution imaging in semi-transparent samples.

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