VIDEO-RATE, MULTI-COLOR STRUCTURED ILLUMINATION MICROSCOPY WITH GPU-BASED, IMMEDIATE IMAGE RECONSTRUCTION

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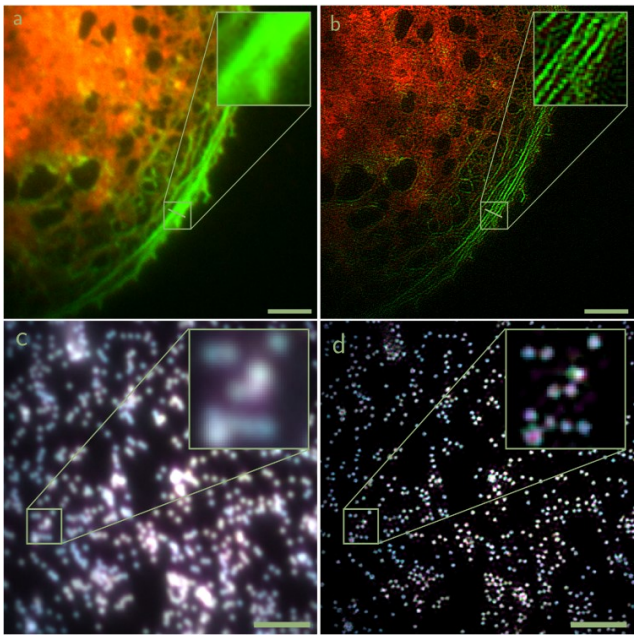
Structured illumination microscopy (SIM) achieves a 2x resolution gain by obtaining 9 to 15 raw images at wide-field light levels. Nowadays, advances in sCMOS cameras and spatial light modulators allow to build cost-effective SIM microscopes that permit multi-color, video-rate fluorescence imaging at super-resolution. These machines are especially suited for live-cell observation, where fast dynamic processes have to be captured with both, high temporal and super-resolved spatial resolution.



Video-rate SIM at 25 reconstructed multicolor frames per second, obtained at 1ms exposure time on our home-built SLM-based SIM setup, **reconstructed in real-time** with <500ms delay between data acquisition and reconstruction display. LSEC stained for plasma membrane (red, CellMask Orange) and actin (green, Alexa Fluor 488) in wide-field (a) and SIM (b) mode. Tetraspeck 200nm fluorescent beads, exited at 647nm (magenta), 568nm (yellow) and 488nm (cyan), in wide-field (c) and SIM (d). Scalebar 2.5µm, insets 1.6µm.

Here, we present our approach of both, a **high-speed 2D SIM microscope** (based on [1]) and a fully **integrated SIM image reconstruction software** (based on our open-source fairSIM project [2]). Our microscope allows for video-rate imaging at up to 3 colors, with frame rates ranging from **25fps** (3-color, 40µm² FOV) to 61 fps (2-color, 20µm² FOV). Our SIM image reconstruction software has been enhanced with **GPU-support** and on-the-fly data processing. For the first time, this provides live (< 500ms delay), multicolor SIM super-resolution imaging of the sample. This opens up many new possibilities, such as scanning large areas in super-resolution quickly, optimizing imaging conditions on the fly, and reacting to dynamic processes in real-time.

Both our software enhancements and schematics of our microscope will be freely available as part of the fairSIM.org project. Our software is compatible with a wide range of home-built and commercial SIM microscopes, so live-view capabilities can be added to these machines.

[1] H. Walther, M. Kielhorn, R. Förster, A. Jost, K. Wicker, and R. Heintzmann. *fastsim: a practical implementation of fast structured illumination microscopy*. Methods and Applications in Fluorescence, 3(1):014001, 2015.
[2] M. Müller, V. Mönkemöller, S. Hennig, W. Hübner, and T. Huser. *Open-source image reconstruction of super-resolution structured illumination microscopy data in ImageJ*. Nature Communications, 7, 2016.