nature research

https://doi.org/10.1038/s41596-020-00408-x

Supplementary information

Doubling the resolution of a confocal spinning-disk microscope using image scanning microscopy

In the format provided by the authors and unedited

Supporting Information

Doubling the Resolution of a Confocal Spinning-Disk Microscope using Image Scanning Microscopy

Shun Qin[1*], Sebastian Isbaner[1*], Ingo Gregor[1], and Jörg Enderlein[1,2]

[1] Third Institute of Physics – Biophysics, Georg August University, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany.

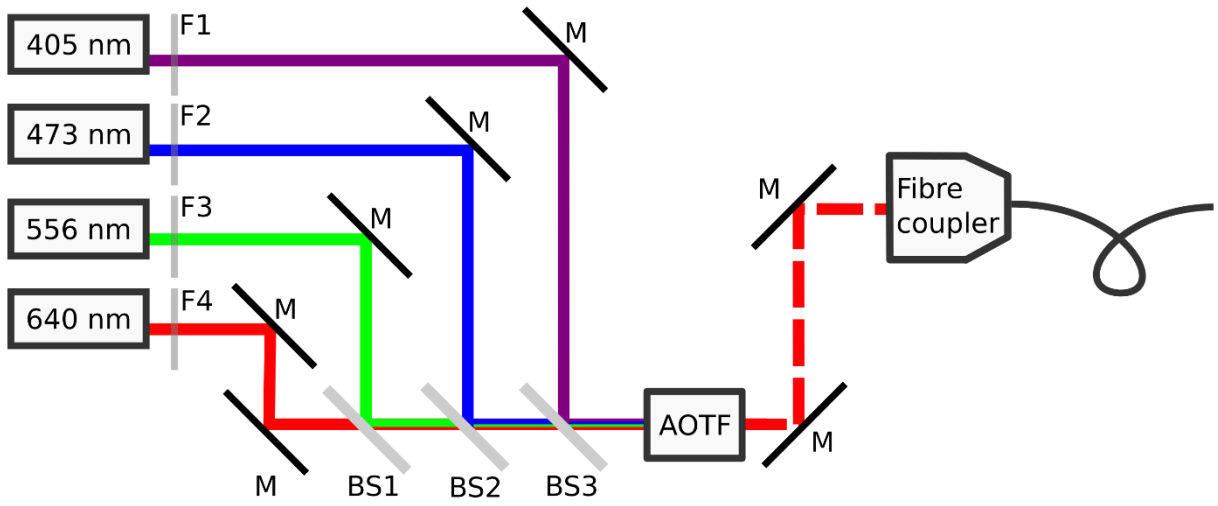
[2] Cluster of Excellence ''Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells'' (MBExC), Georg August University, Göttingen, Germany.

Correspondence should be addressed to J.E. (jenderl@gwdg.de)

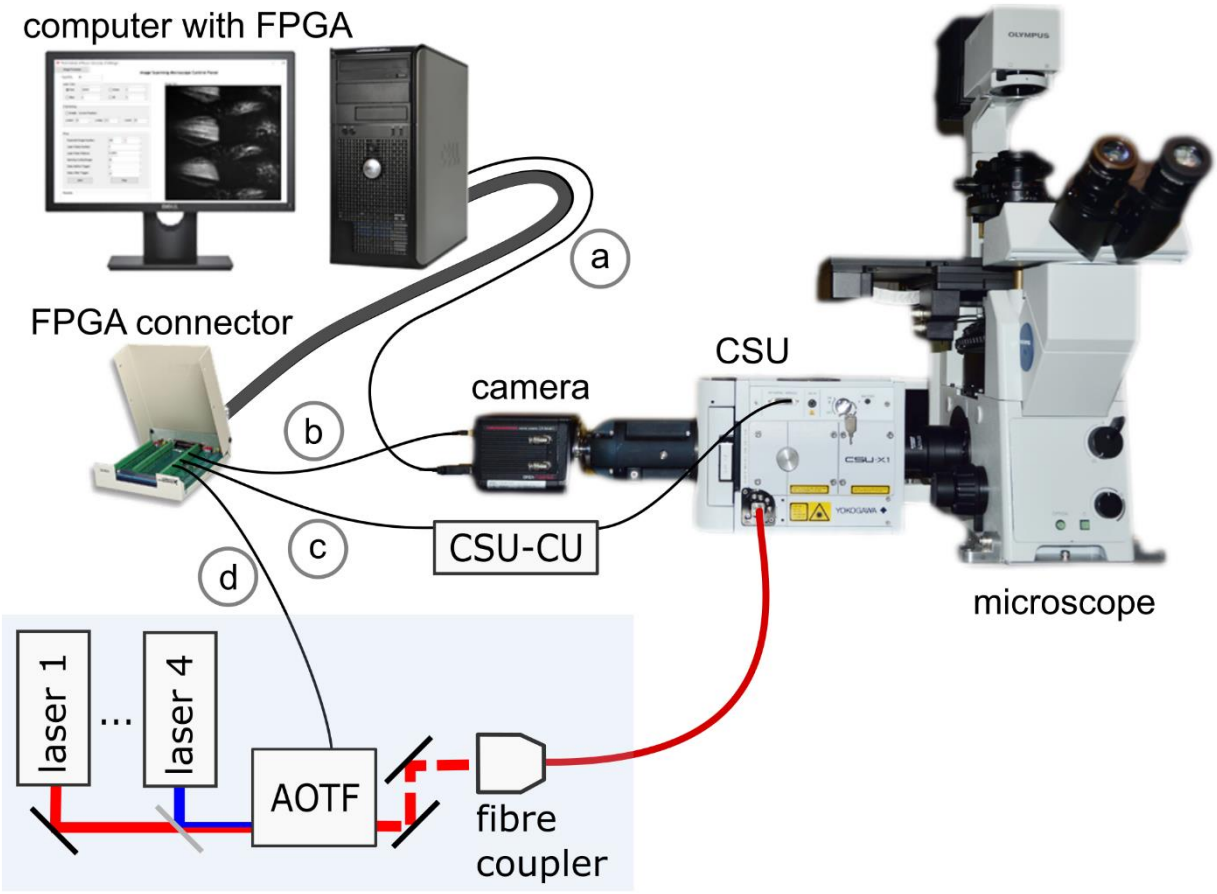
*These authors contributed equally to this work

Supplementary Note 1: Trigger Signal

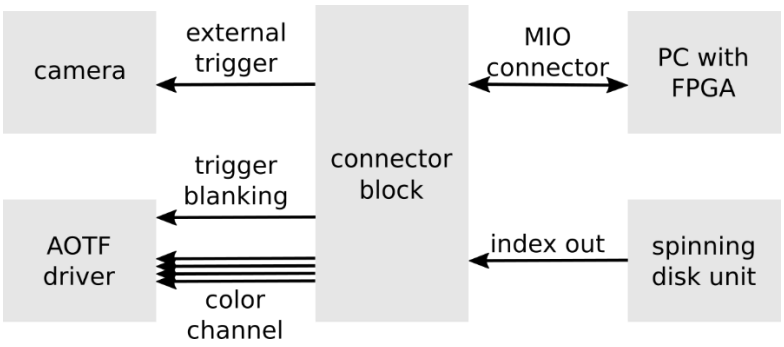
The trigger signals are used by the FPGA to control the stroboscopic illumination of the lasers and the image acquisition of the camera. The output of our FPGA is either a digital signal (LOW and HIGH according to CMOS specifications) or an analog signal (between -10V and +10V). The stroboscopic illumination is controlled via a single blanking signal with HIGH indicating when the AOTF should switch on. The channel and its intensity of the AOTF is controlled via four analog outputs with voltage value between 0 and 10 V (0 V = channel off, >0-10 V controls the RF power of the AOTF). Alternatively, four digital signals can be used to directly modulate the light source (these four channels have the same output as the one for the blanking line, but is LOW when the corresponding channel in the plugin is switched off). In addition to illumination, also image acquisition by the camera is controlled by an FPGA trigger signal. The HIGH level indicates the start of image acquisition, and the duration of the HIGH indicates the duration of exposure. The time between two HIGHs can be used for read-out. During this time, the laser triggering is inactive.



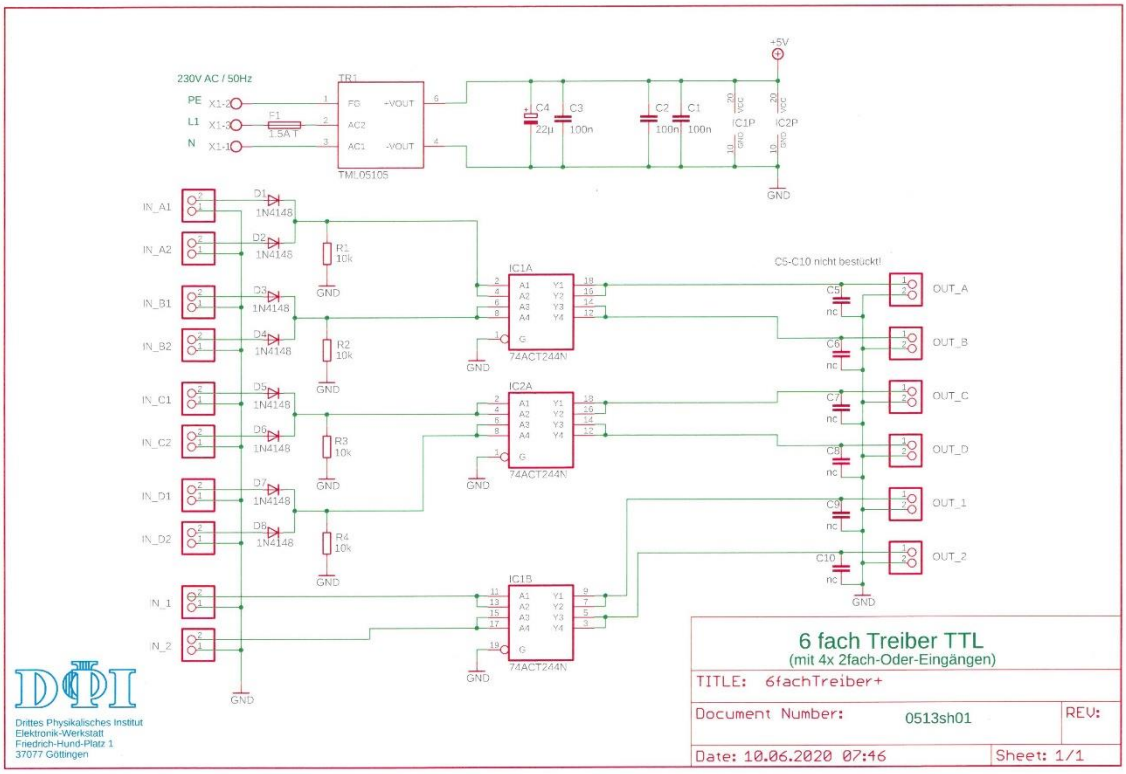
Supplementary Figure 1: Excitation path: The four lasers, equipped with a clean-up filter (F1-F4), can be adjusted by one mirror (M) and one dichroic mirror (BS1-3**)** each. The acousto-optic tuneable filter (AOTF) is used for wavelength selection and stroboscopic illumination. The beam is coupled into an optical fibre by means of two mirrors.



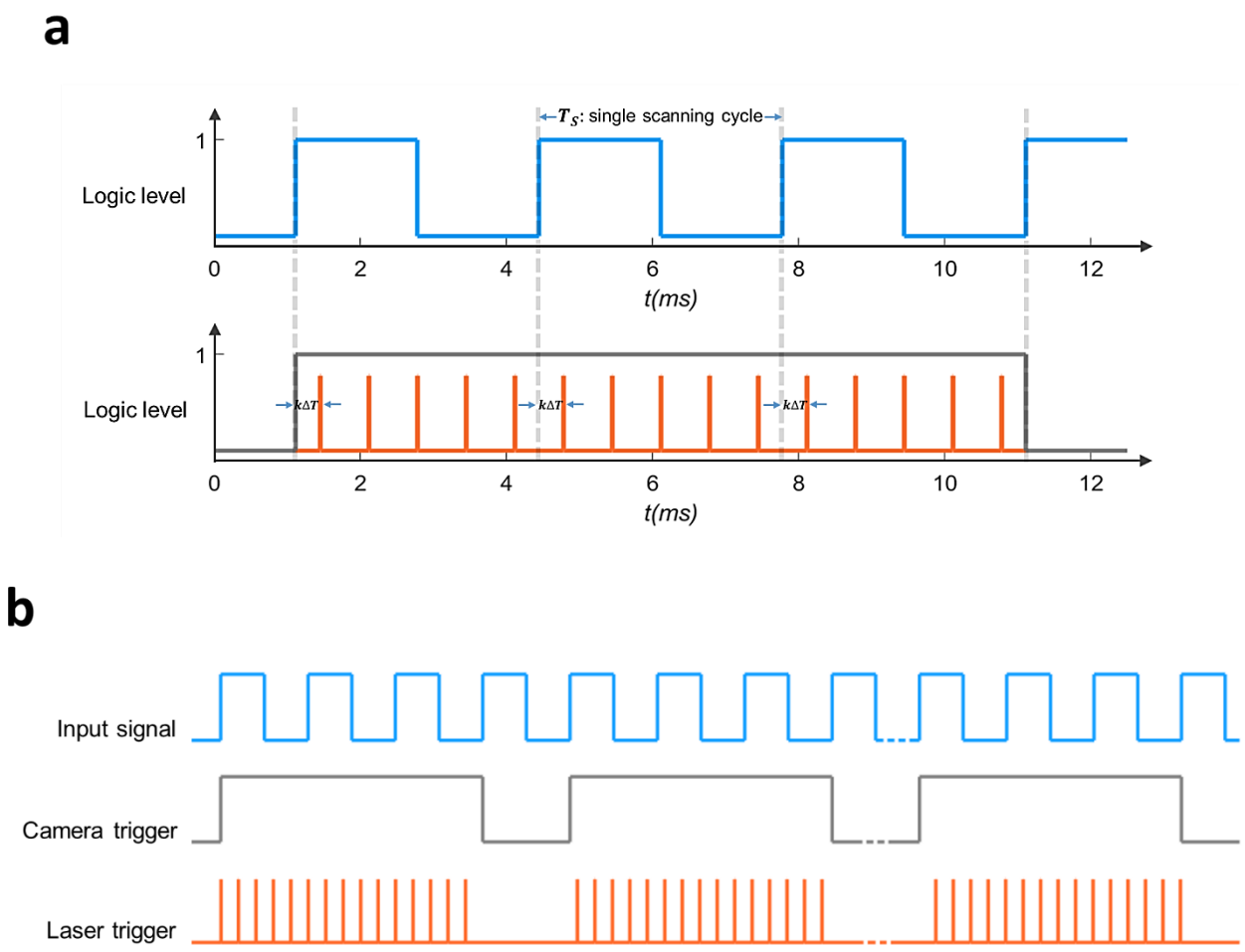
Supplementary Figure 2: CSDISM setup. A computer with an FPGA controls running Micromanager with the CSDISM plugin is connected to the camera via USB (a). The FPGA connector block triggers the camera (b) and receives the timing signal of the confocal spinning disk unit (CSU) via the CSU control unit (CSU-CU) (c). At the same time, the FPGA controls the AOTF (d) via the AOTF driver to achieve stroboscopic illumination.



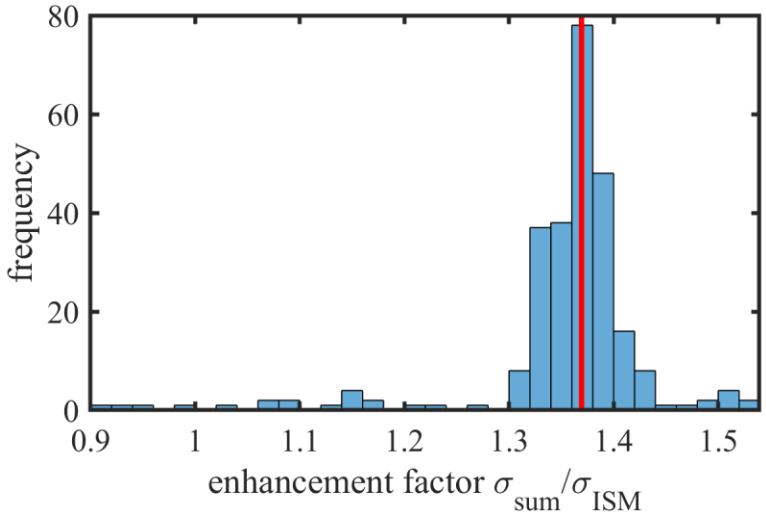
Supplementary Figure 3: Connections of the FPGA card to the setup components. The FPGA is connected via a MIO connector to the connector block (top right). The incoming signal of the spinning disk (bottom right) is used to synchronize the image acquisition of the camera (top left) and the stroboscopic illumination via the AOTF (bottom left). If the lasers allow fast electric modulation of their intensity, they can be connected directly to the FPGA instead of an AOTF. Note that in this case, the channels on the connector block change, see main text for further details.



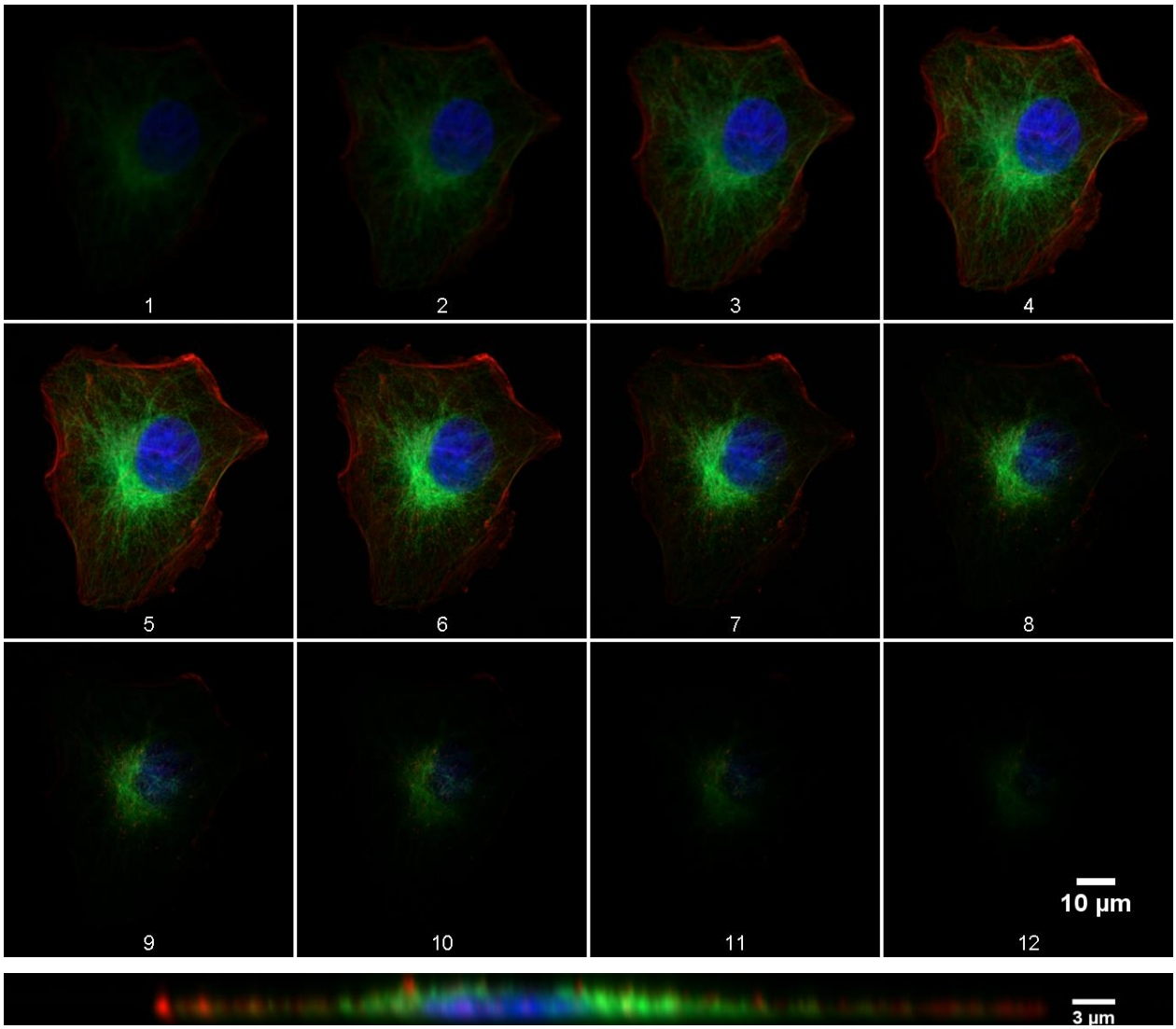
Supplementary Figure 4: Electrical circuit diagram of the custom TTL 5V amplifier.



Supplementary Figure 5: Camera and laser trigger signal for recoding the $\mathbf{k^{th}}$ frame (a): input signal in blue comes from the spinning disk control unit (CSU). Each rising edge indicates the start of a new scan cycle. (b) Camera and laser trigger signal for taking the first, second and the last frame. In this case, three scan cycles are used to take one raw image. Five laser triggers are generated during each cycle, and the sample is illuminated by a total of 15 laser pulses during one exposure time. If observed through an oscilloscope, the laser triggers will shift from the rising edge towards the falling edge of the camera trigger during image acquisition.



Supplementary Figure 6: Quantification of resolution enhancement by ISM. We use the image of beads as shown in Figure 4 and fit a 2D Gaussian to each bead in the ISM image and the sum image. The ratio of the width of the Gaussian is shown here as the "enhancement factor". The mean (red line) is close to the ideal enhancement of $\sqrt{2} \approx 1.41$ (which holds for the FWHM without deconvolution). The experimental enhancement factor is reduced because the theoretical value does not take the Stokes shift into account. Figure replicated from Isbaner, S. Extending Resolution in All Directions: Image Scanning Microscopy and Metal-induced Energy Transfer. (2019).



Supplementary Figure 7: 3D multi-colour CSDISM imaging of a fixed cell. The top images show individual slices with 300 nm distance between focal planes. The bottom image is a YZ section through the centre of the image. The colours correspond to: blue – nucleus (DAPI), green – tubulin (FITC-alpha-tubulin), and red – actin (Atto 550-phalloidin). The image was acquired using the Hamamatsu Orca Flash camera and a 60x water immersion objective (UPLSAPO 1.2 NA, Olympus). Figure replicated from Isbaner, S. Extending Resolution in All Directions: Image Scanning Microscopy and Metal-induced Energy Transfer. (2019).

Figure	Number of images	Number of pulses N_p	Laser Pulse Width	Spinning Cycles/Image	Camera
Fig. 3	250	4	6 µs	200	EMCCD
Fig. 4 a)	250	4	6 µs	60	EMCCD
Fig. 4 c)	250	4	6 µs	200	sCMOS 2x2 binning
Fig. 4 e)	250	4	6 µs	30/10/30 (excitation with 405/473/556 nm)	EMCCD

Supplementary Table 1: CSDISM parameters for Figures 3 and 4 in the main text.