Super-resolution imaging of neuronal structure with structured illumination microscopy – Supplementary information

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Supplementary note 1: SIM system components, characteristics of the camera, microdisplay, and light source; parameters of the imaging data.

Table S1. Main components of the SIM system

Component	Vendor	Vendor website	Part number
Microdisplay	Forth Dimension Displays	https://www.forthdd.com/	SXGA-3DM
Camera	Andor	https://andor.oxinst.com/	Zyla 4.2+
Light source	Lumencor	https://lumencor.com/	Spectra-X
Microscope	Olympus	https://www.olympus-lifescience.com/en/	IX83
Stage	Applied Scientific Instrumentation	https://www.asiimaging.com/	PZ-2500FT and MS-2000-WK
Mounting for microdisplay	Thor Labs	https://www.thorlabs.com/	PT3
Polarizing beam splitter	Thor Labs	https://www.thorlabs.com/	CCM1-PBS251
Optical lens (coupling from liquid light guide)	Thor Labs	https://www.thorlabs.com/	AC254-050-A-ML
Optical lens (illumination tube lens)	Olympus	https://www.olympus-lifescience.com/en/	SWTLU-C
Filters	Chroma	https://www.chroma.com/	475/34 ex. filter T495lpxr dichroic ER525/50m em. filter
Input/output card	Measurement Computing	https://www.mccdaq.com/	PCIM-DDA06/16

Table S2. Camera parameters

Manufacturer	Andor
Part number	ZYLA-4.2P-CL10
Sensor type	Front illuminated scientific CMOS
Sensor size	13.3×13.3 mm
Interface	camera link
Quantum efficiency	82% at 555 nm
Read noise	0.9 e^-
Data range	16-bit
Pixel size	6.5 µm
Well depth	30,000 e^-
Resolution	2048×2048 pixels
Maximum frame rate	100 FPS
Readout method	rolling shutter

Table S3. Light source parameters

Manufacturer	Lumencor
Part number	Spectra-X
Output Wavelengths, nm	395, 440, 470, 550, 575, 640, 730
Turn-on time	10 µs
Turn-off time	2 µs

Table S4. Microdisplay parameters

Manufacturer	Forth Dimension Displays
Part number (system)	SXGA-3DM
Part number (microdisplay)	M249
Part number (interface)	R12
Microdisplay type	Ferroelectric liquid crystal on silicon (FLCOS)
Wavelength range	430-700 nm
Resolution	1280×1024 pixels
Switching speed	40 µs
Active area	17.43 mm×13.95 mm 22.3 mm diagonal
Pixel size	13.62 µm
Inter pixel gap	0.54 µm
Maximum frame rate	3.2 KHz binary images
Fill factor	93%
Reflectance in 'ON' state	60%
Onboard memory	768 binary images

Table S5. Camera parameters for the machine vision camera used (only) in Figs. S4 and S5.

Manufacturer	Teledyne FLIR
Part number	BFS-U3-244S8M-C
Camera model	FLIR blackfly S
Sensor type	Back illuminated CMOS
Sensor type	Sony IMX540
Sensor size	19.36 mm diag.
Interface	USB3
Quantum efficiency	69% at 525 nm
Read noise	2.31 e^-
Data range	12-bit
Pixel size	2.74 µm
Well depth	9648 e^-
Resolution	5320×4600 pixels
Maximum frame rate	15 FPS
Readout method	global shutter

Table S6. Parameters of the imaging data

	Camera	Objective (Olympus)	Exposure time	SIM pattern*	Z-spacing and num. slices	Acquisition time Processing time
Fig. 2	Andor Zyla	UPLAPO 10×/0.4 NA water immersion	100 ms	2-10-1-12	12 slices Spacing 20 um (10x6 pano.)	5:30 to aquire 15:35 proc. time
Fig. 3&4	Andor Zyla	UPLSAPO 100×/1.4 NA oil immersion	300 ms	2-10-1-12	126 slices Spacing 200 nm	19 min to acquire 36 min proc. time
Fig. 5	Andor Zyla	UPLSAPO 100×/1.4 NA oil immersion	800 ms	2-10-1-12	61 slices 200 nm spacing	22 min to acquire 17 min proc. time
Fig. 6	Andor Zyla	UPLXAPO 60×/1.42 NA oil immersion	100 ms (40% power)	2-10-1-12	566 slices 200 nm spacing	62 min to acquire 73 min proc. time
Fig. 7a	Andor Zyla	UPLSAPO 20×/0.75 NA air	150 ms	Various	35 slices 5 um spacing	Various
Fig. 7b	Andor Zyla	UPLSAPO 100×/1.4 NA oil immersion	250 ms	1-2-1-3	51slices 200 nm spacing	2 min to acquire 3 min proc. time
Fig. 7c	Andor Zyla	UPLSAPO 100×/1.4 NA oil immersion	250 ms	1-2-1-3	21 slices 200 nm spacing	2 min to acquire 1 min proc. time
Fig. 7d	Andor Zyla	UPLSAPO 100×/1.4 NA oil immersion	250 ms	2-10-1-12	21 slices 200 nm spacing	5 min to acquire 4 min proc. time
Fig. S1&S2	Andor Zyla	UPLSAPO 100×/1.4 NA oil immersion	400 ms	1-9-1-10	47 slices 200 nm spacing	26 min to acquire 17 min proc. time
Fig. S4	FLIR Blackfly S	UPLSAPO 60×/1.35 NA oil immersion	ND**	1-9-1-10	69 slices 145 nm spacing	ND** 17 min proc. time
Fig. S5	FLIR Blackfly S	UPLAPO 10×/0.4 NA Oil immersion	ND**	1-10-1-11	9 slices 15 um spacing	ND** 11 min proc. time
Fig. S6	Andor Zyla	UPLXAPO 60×/1.42 NA oil immersion	100 ms (20% power)	2-10-1-12	No Z in this experiment	30 min to acquire 6 min proc. time

***** A SIM pattern of 2-10-1-12 denotes 2 columns of pixels ON, 10 columns of pixels OFF, an inter-pattern shift of 1 pixel, and a total of 12 patterns in the set. Similarly pattern 1-9-1-10 denotes 1 column of pixels ON, 9 columns of pixels OFF, a shift of 1 pixel and a total of 10 patterns in the set.

** Some of the metadata was lost for these experiments; the exposure time was ~200 ms. Acquisition times were about 10-15 minutes.

Supplementary note 2: supplementary figures showing additional images of cortical neurons and resolution analysis by Fourier ring correlation (FRC) and power spectral density (PSD) methods.

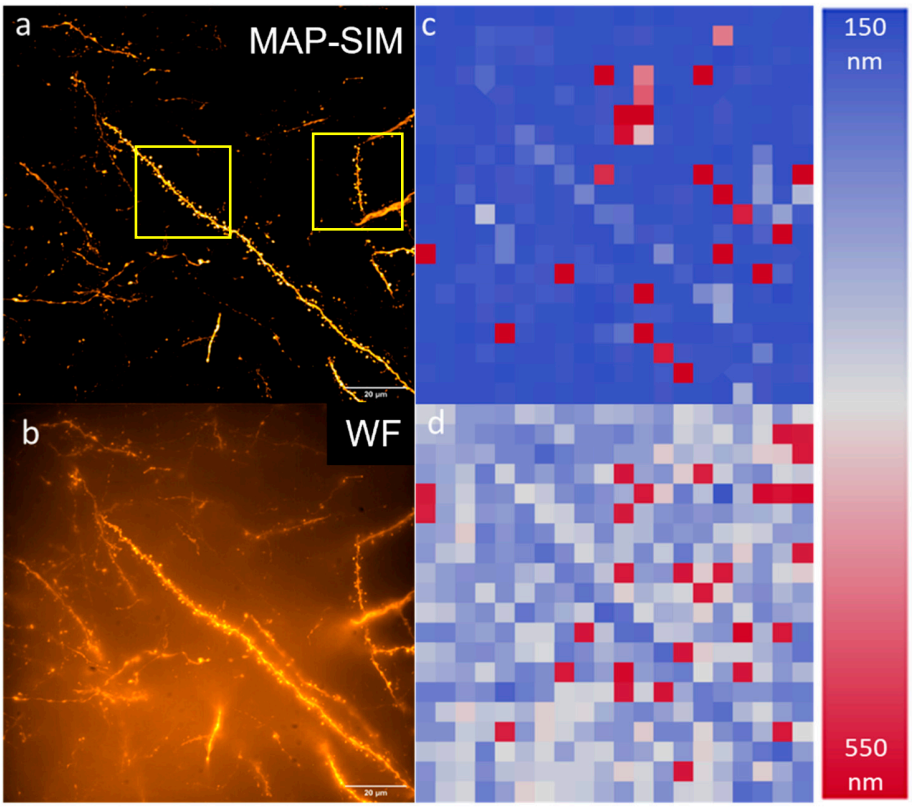

Figure S1: Cortical neuron. Imaged with (a) MAP-SIM, (b) conventional widefield, (c) FRC measurement of MAP-SIM, (d) FRC measurement of WF. FRC measurements [60] were performed using NanoJ-squirrel [61].

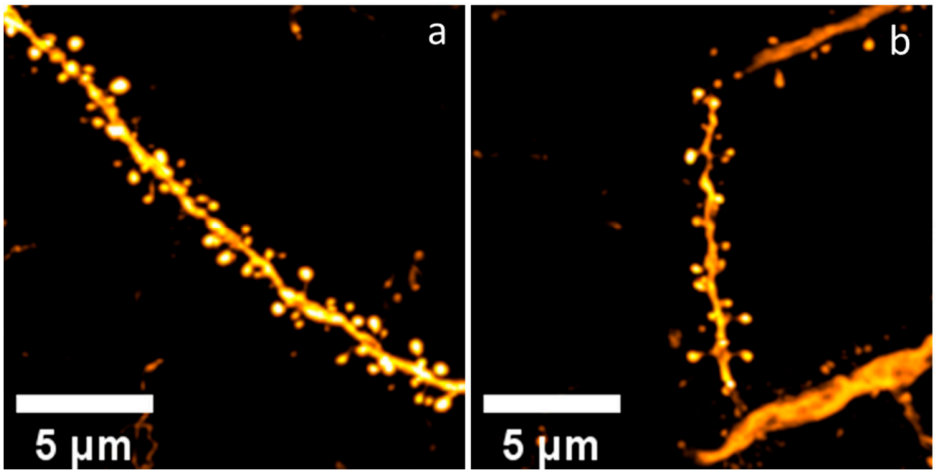

Figure S2: Enlarged views. (a, b) Areas indicated by yellow boxes in Figure S1a.

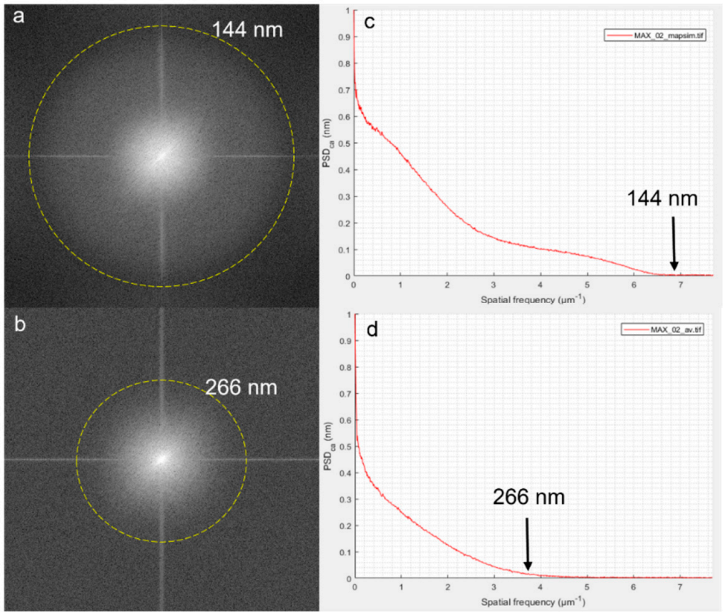

Figure S3: Resolution analysis. (a, c) FFT of Figure S1a and corresponding power spectral density measurement, (b,d) FFT of Figure S1b and corresponding power spectral density (PSD) measurement. PSD was measured using SRMeasureToolbox[50].

Supplementary note 3: supplementary figures showing images of cortical neurons and neurons of the midbrain imaged with a machine vision camera.

Figures S4 and S5 show SIM images of a cortical neuron, and neurons of the midbrain, acquired with a machine vision camera at two different magnifications. This 24.5 megapixel camera is low cost compared to the Andor Zyla camera, but has adequate performance for this application. Table S5 shows parameters of this particular camera. This data illustrates how the SIM system described here can be adapted for use with lower cost components, making this system more accessible to a larger number of investigators. The complete SIM system described here is much lower in cost than other optical sectioning microscopes such as point scanning confocal or spinning disk systems, while also offering super-resolution.

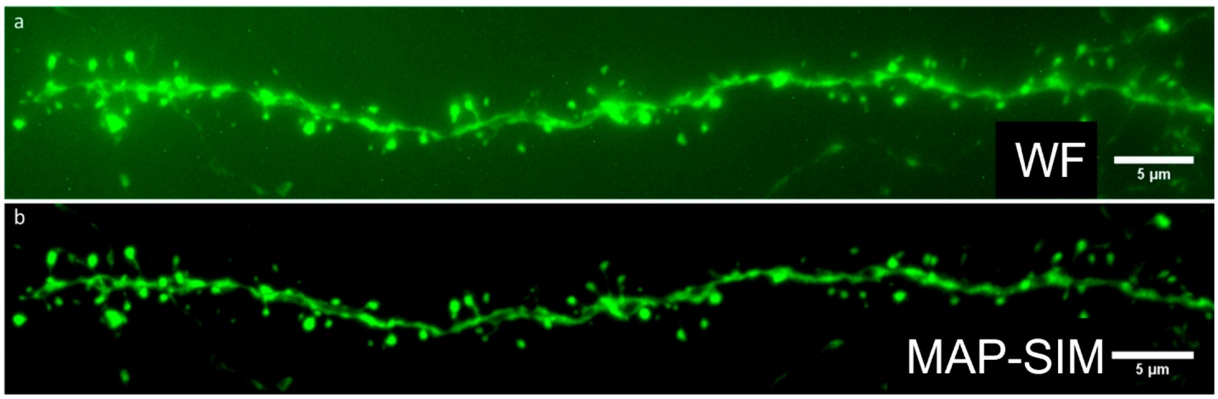

Figure S4: Cortical Neuron. Imaged with (a) conventional widefield and (b) MAP-SIM. Objective: Olympus UPLSAPO 60×/1.35 NA oil immersion. Camera: FLIR Blackfly S.

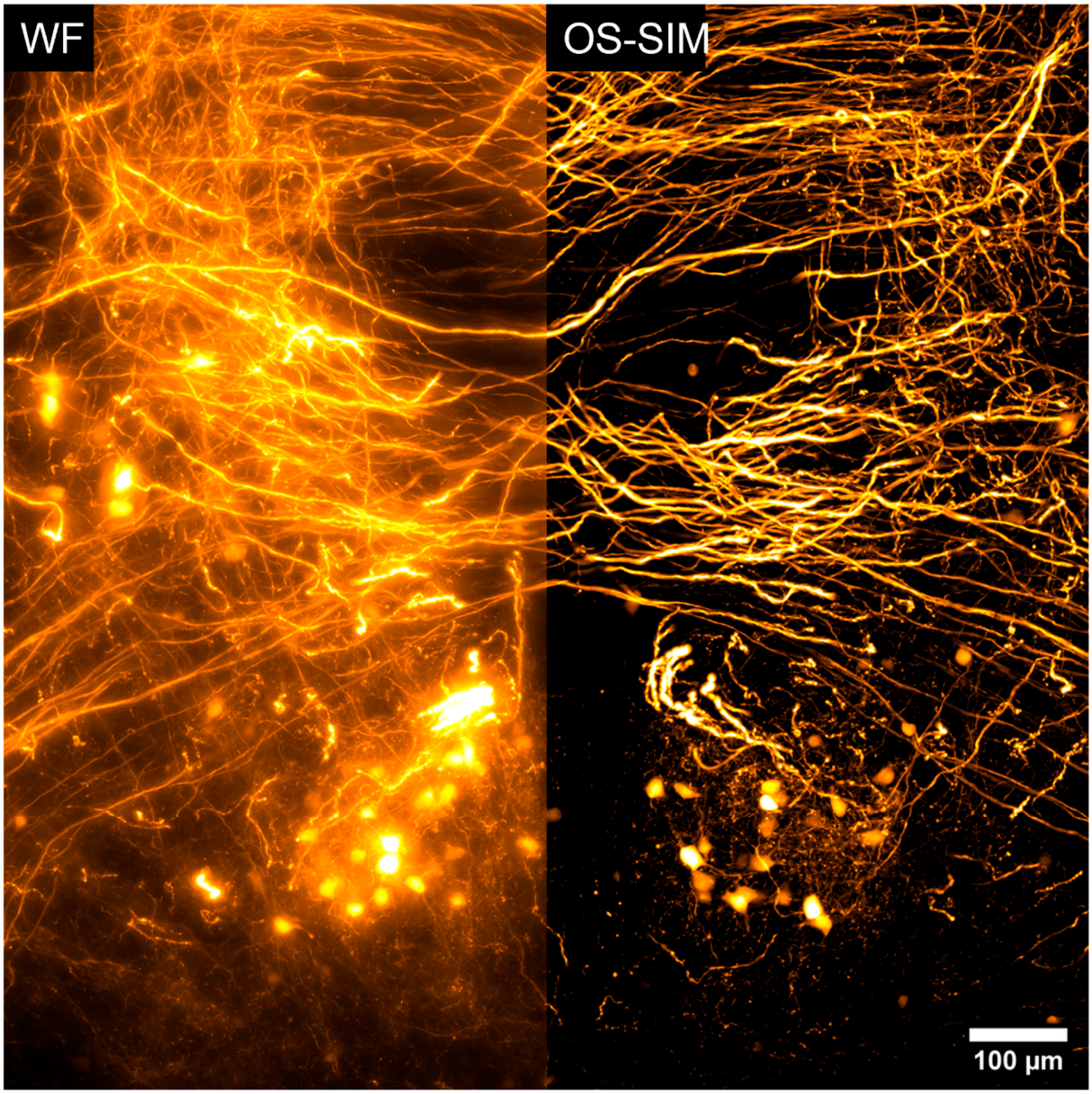

Figure S5: Neurons of the midbrain. Imaged with (left) WF, and (right) OS-SIM. Objective: Olympus UPLANAPO 10×/0.4 NA oil immersion. Camera: FLIR Blackfly S.

Supplementary note 4: supplementary figure showing analysis of photobleaching during SIM imaging.

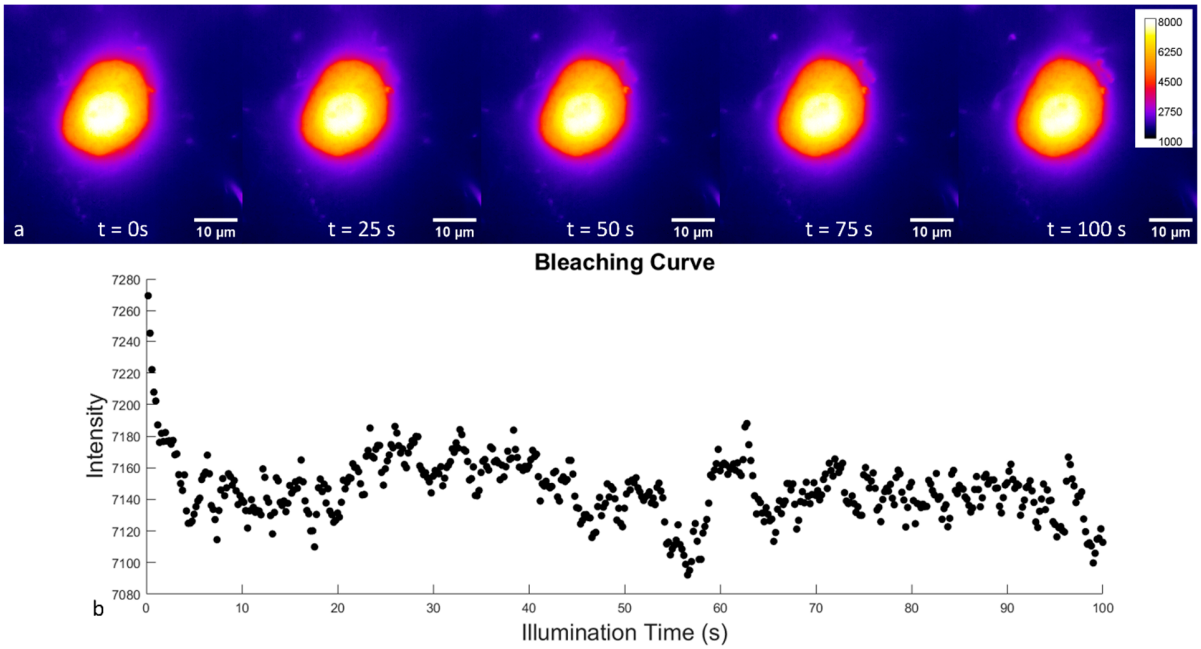

Figure S6: Photobleaching analysis. (a) Images of a neuron over time during continuous exposure using the 2-10-1-12 pattern. Images were taken using a 60× objective with an exposure time of 100 ms. (b) Average intensity over time of the boxed area in Figure S6a. Intensity decreased only 1.7% after 100 s of illumination.

Supplementary note 5: additional details about the setup used in SIM imaging.

While the camera and XYZ stage receive signals directly from Andor IQ software and an input/output computer card (Measurement Computing DDA06/16), the illumination signals which the software generates must be altered before being sent to the light source. Firstly, the microdisplay used in our setup will not produce an image on the sample if it is illuminated with a constant light source. Rather, a meaningful illumination pattern will only form if the light source is synchronized with an enable signal output from the microdisplay control board. Therefore, the channel selection signals output by IQ software and the I/O card are first modulated with the microdisplay enable signal. This is performed by the leftmost AND gates pictured in Figure S7 (see also Figure 1 of the main text). Additionally, to reduce unnecessary light exposure to the sample, the light source is shut off whenever the camera sensor is not being exposed. This is accomplished by performing a second logical AND of the result of the previous AND with the 'FIRE' signal output from the camera. This process is illustrated in Figure S7.

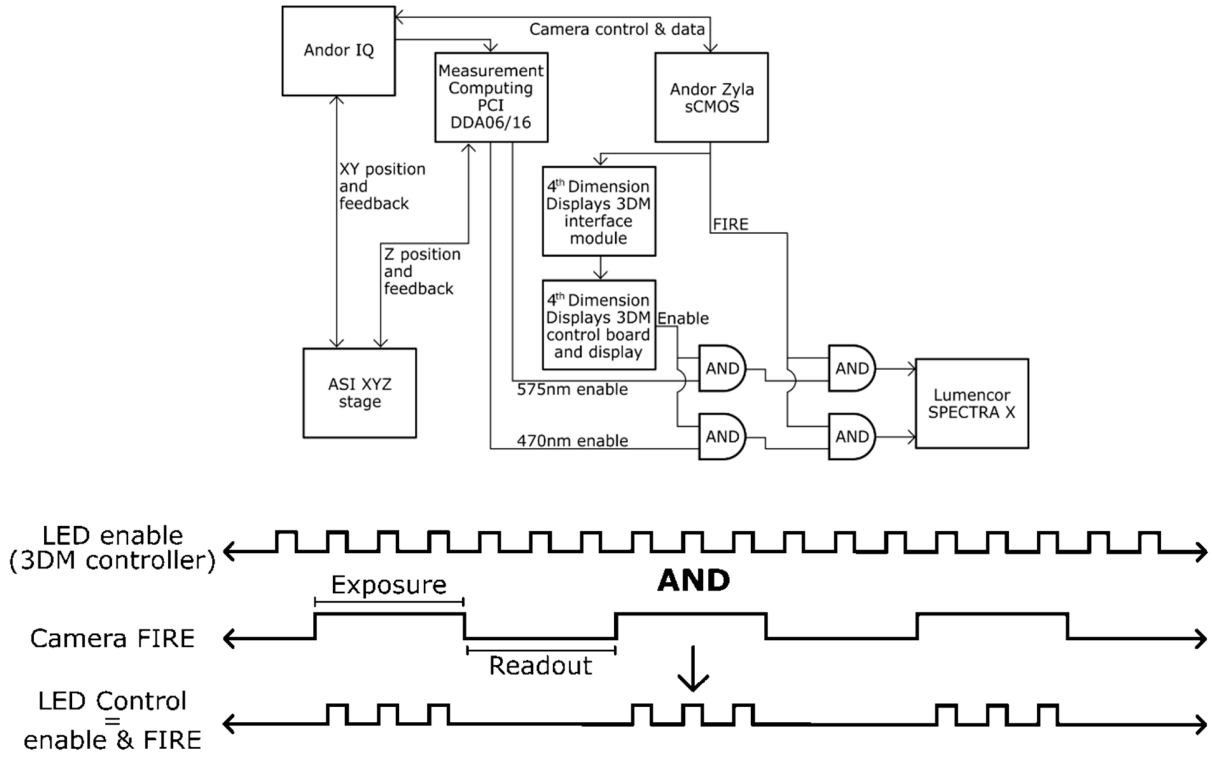

Figure S7: SIM system details. (above) connection diagram (below) schematic of the timing scheme.

Figure S8a shows a diagram of the optical system. Figure S8b shows the principle of the microdisplay operation. The microdisplay is a ferroelectric, liquid crystal-on-silicon device which functions as an array of quarter wave plates with a reflective backing. Each pixel of the array can be individually turned ON or OFF. In the ON state, the quarter wave plate in that pixel converts linearly polarized light to circular polarization. Vertically polarized light reflects off of a polarizing beam splitter cube and into the device where it passes through the quarter wave plate, reflects off the mirror-like backing, and passes a second time through the quarter wave plate. This has the effect of converting vertically polarized light to horizontally polarized light, which then passes through the polarizing beam splitter cube and enters the microscope. Use of an epifluorescence microscope with filter cubes in a rotating turret lets one easily perform multi-color imaging. Multi-band filter sets and electronically controlled light sources allow multi-color structured illumination microscopy with no moving parts. 3D imaging is accomplished slice-by-slice by changing the sample focus. We have successfully used microscopes from Olympus (IX71, IX83) and Leica (DMI8) in similar SIM systems.

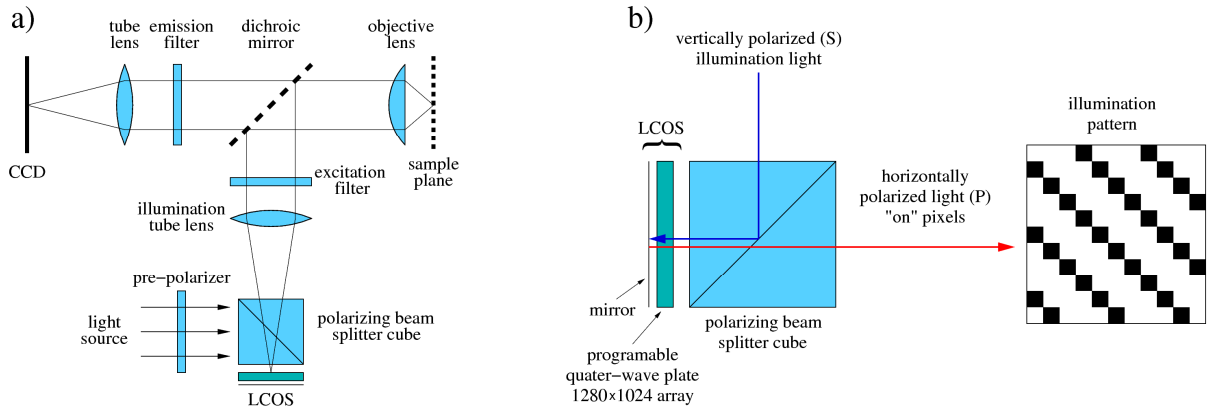

Figure S8: SIM system diagrams. (a) simplified diagram of the system (b) illustration of microdisplay operation.

Supplementary note 6: SIMToolbox resources.

SimToolbox homepage: https://github.com/simtoolbox
SIMToolbox[45] was used to process all of the data in this paper. SIMToolbox is an open-source, modular set of functions for MATLAB equipped with a user-friendly graphical interface and designed for processing two-dimensional and three-dimensional data acquired by structured illumination microscopy. Both optical sectioning and super-resolution applications are supported. The software is also capable of maximum a posteriori probability image estimation (MAP-SIM), the method used in this paper.

Test files: https://zenodo.org/record/4044159#.YOYRe35MEuU
This site contains data for testing SIMToolbox and for comparison with data acquired with other SIM systems. The available data includes images acquired with our SIM system, and also data acquired with Zeiss and Nikon SIM systems.

SIMToolbox documentation: https://github.com/simtoolbox/Documentation
This site contains documentation related to SIMToolbox, including quick-start guides for processing data acquired with our system or with Nikon systems.

Supplementary references

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61. Culley, S.; Albrecht, D.; Jacobs, C.; Pereira, P.M.; Leterrier, C.; Mercer, J.; Henriques, R. Quantitative mapping and minimization of super-resolution optical imaging artifacts. *Nat. Methods* **2018**, *15*, 263–266.
50. Pospíšil, J.; Fliegel, K.; Klíma, M. Assessing resolution in live cell structured illumination microscopy. In Proceedings of the Proceedings of SPIE - The International Society for Optical Engineering; Páta, P., Fliegel, K., Eds.; SPIE, 2017; Vol. 10603, p. 39.
45. Křížek, P.; Lukeš, T.; Ovesný, M.; Fliegel, K.; Hagen, G.M. SIMToolbox: A MATLAB toolbox for structured illumination fluorescence microscopy. *Bioinformatics* **2015**, *32*, 318–320.