Supplementary Materials for

A versatile twin-microscope system for light-sheet imaging

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Captions for Movies S1 to S3

Supplementary Note 1

Other Supplementary Materials for this manuscript include the following:

Movies S1 to S3

SUPPLEMENTARY TABLE

TABLE SI. List of main flex-SPIM components

Subsystem/ module	Component	Catalogue number	Vendor	Notes
	5 foot × 10 foot, anti-vibration optical table	INTEGRITY 4 VCS 510-8	Newport	
CW Laser bank	OBIS 445 nm LX	1185051	Coherent	Power: 75 mW
	OBIS 488 nm LX	1220123	Coherent	Power: 150 mW
	OBIS 561 nm LS	1280720	Coherent	Power: 150 mW
	OBIS 647 nm LX	1196627	Coherent	Power: 120 mW
	f = 20 mm, 12.5 mm diameter lens	47-661	Edmund Optics	Beam expansion
	f = 8 mm, 12.24 mm diameter lens	C240TME-A	Thorlabs	
	25 mm diameter broadband mirror	87-371	Edmund Optics	Beam combining
	Di02-R561 dichroic beamsplitter	Di02-R561-25x36	Semrock	Beam combining
	LM01-503-25 LaserMUX dichroic beamsplitter	LM01-503-25	Semrock	Beam combining
	LM01-466-25 LaserMUX dichroic beamsplitter	LM01-466-25	Semrock	Beam combining
	1 inch diameter broadband dielectric mirrors	BB1-E02	Thorlabs	Broadband (400-750 nm) dielectric beam steering mirror
	30 mm cage cube-mounted variable beamsplitter	VA5-PBS251	Thorlabs	Visible (420-680 nm) polarization optics
	0.5 inch diameter mounted achromatic half-wave plate	AHWP05M-600	Thorlabs	Visible (400-800 nm) polarization optic
	AOTF	AOTFnC-400.650-TN	AA Quanta Tech, Optoelectronic	Tellurium dioxide crystal Number of channels: 8 Wavelength range: 400-650 nm Transmission: > 90% Aperture: 3 mm^2 Spectral resolution: 1-4 nm Tuning time: < 4 μs
	Driver for AOTF	MPDS8C-D66-22-74.158	AA Quanta Tech	Number of channels: 8 Communication: USB, RS232, RC03 Extinction ratio: 120 dB
	RF cable SMA connectors	CBL-SAM200SAM-RG223	AA Quanta Tech	
	STAB, cable, SMC connectors	CBL-SCF200SCF-RG316	AA Quanta Tech	
	Insight DS+	90044047	Spectra Physics	Tuning range: 680-1300 nm

Subsystem/ module	Component	Catalogue number	Vendor	Notes
NIR femtosecond-pulsed laser				Tuning. range: 680-1300 nm Repetition rate: 80 ± 0.5 MHz
	30 mm cage cube-mounted variable beamsplitter	VA5-PBS252	Thorlabs	NIR (690-1000 nm) polarization optics
	1 inch diameter broadband dielectric mirrors	BB1-E03	Thorlabs	Broadband (750-1100 nm) dielectric beam steering mirror
	E-O Modulator 2.7mm Aperture	350-80-02 KD*P	Conoptics	
	Galilean beam expander	BE02-05-B	Thorlabs	2×-5× optical beam expander
	800 nm long-pass filter, 1 inch diameter	FEL0800	Thorlabs	
Illumination-scanning optics	24 inch × 36 inch optical breadboard	B2436F	Thorlabs	
	66 mm construction rail	XT66-500	Thorlabs	Periscope
	1 inch kinematic mirror mount	KM100	Thorlabs	
	45° elliptical mirror mount	H45E1	Thorlabs	
	VIS elliptical mirror	BBE1-E02	Thorlabs	
	NIR elliptical mirror	BBE1-E03	Thorlabs	
	1 inch pedestal post, 1inch long	RS1P8E	Thorlabs	
	clamping platform for 66 mm rail	XT66C4	Thorlabs	
	0.5 inch diameter silver mirror	PF05-03-P01	Thorlabs	Beam steering mirror
	0.5 inch diameter mounted achromatic half-wave plate	AHWP05M-600	Thorlabs	Visible (400-800 nm) polarization optic
	30 mm cage cube-mounted variable beamsplitter	VA5-PBS252	Thorlabs	NIR (690-1000 nm) polarization optics
	2D scanning galvo mirror positioning system	GVSM002	Thorlabs	
	Mounting adapter for 2D galvo system	GCM102	Thorlabs	
	f = 150 mm, 50 mm diameter lens	VIS-NIR 49-391-INK	Edmund Optics	Scan lens (achromatic doublet)
	f = 200 mm, 50 mm diameter lens	VIS-NIR 49-392-INK	Edmund Optics	Tube lens
	5×, 0.10 NA, 23 mm WD objective lens	LMPLN5XIR LWD M PLAN	Olympus	Excitation objective lens
Detection	20×, 1.0 NA, 2 mm WD objective lens	XLUMPLFLN-W	Olympus	Detection objective lens

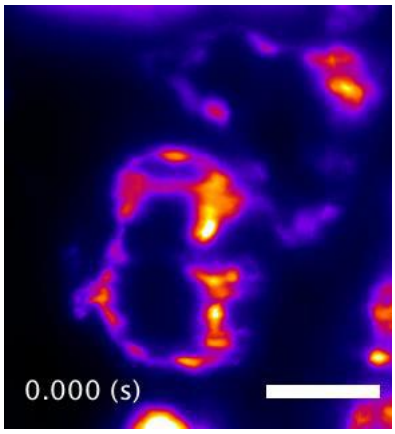
Subsystem/ module	Component	Catalogue number	Vendor	Notes
	Filter wheel	Lambda 10-B	Sutter Instrument	Twin 1: 32 mm diameter Twin 2: 25 mm diameter
	Filter wheel controller	Lambda 10-3	Sutter Instrument	
	609/54 nm BrightLine single-band bandpass filter	FF01-609/54-32	Semrock	Emission filter set for Twin 1
	680/42 nm BrightLine single-band bandpass filter	FF01-680/42-32	Semrock	
	525/50 nm BrightLine single-band bandpass filter	FF03-525/50-32	Semrock	
	470/28 nm BrightLine single-band bandpass	FF01-470/28-32	Semrock	
	609/54 nm BrightLine single-band bandpass filter	FF01-609/54-25-STR	Semrock	Emission filter set for Twin 2
	680/42 nm BrightLine single-band bandpass filter	FF01-680/42-25-STR	Semrock	
	525/45 nm single-band bandpass filter	FF01-525/45-25-STR	Semrock	
	472/30 nm BrightLine single-band bandpass filter	FF02-472/30-25-STR	Semrock	
	f = 100 mm, 50 mm diameter lens	VIS-NIR 49-284-INK	Edmund Optics	Tube lens (for 11× magnification)
	f = 400 mm, 75 mm diameter lens	VIS-NIR 88-598-INK	Edmund Optics	Tube lens (for 44× magnification)
	ORCA-Flash4.0 V3 Digital CMOS camera	C13440-20CU	Hamamatsu	Twin 1 Number of pixels: 2048 × 2048 Pixel size: 6.5 μm^2 Full resolution frame rate: 100 frames/s (Camera link) 40 frames/s (USB) Quantum efficiency: 82% peak
	ORCA-Flash4.0 V2 Digital CMOS camera	C11440-22CU	Hamamatsu	Twin 2 Similar specifications to camera on Twin 1
Sample mounting	Sample chamber	Custom	Protolabs	Material: Delrin 150 black acetal homopolymer
	Clamping rings	Custom	Protolabs	Material: Delrin 150 black acetal

Subsystem/ module	Component	Catalogue number	Vendor	Notes
				homopolymer; used to clamp glass windows to sample chamber
	Heat exchanger	Custom	Protolabs	Material: Aluminum 6061-T651 or Cooper
	Caddy	Custom	Protolabs	Material: WaterShed XC 11122
	Dive bar	Custom	Protolabs	Material: Stainless steel 316/316L
	Dive bar to goniometer mount	Custom	Protolabs	Material: Stainless Steel 316/316L
	Dual-axis goniometer	GN2/M	Thorlabs	
	Metric baseplate	UBP2/M	Thorlabs	
	Breadboard	MB1012	Thorlabs	For sample stack-up
	High-purity silicone Rubber .010" thick	87315K62	McMaster-Carr	Material: 55A Durometer; gasket used to clamp tightly seal glass windows to sample chamber
	High-purity silicone Rubber .020" thick	87315K63	McMaster-Carr	
	40mm glass coverslips	10200-060	VWR	Used as bottom window for sample chamber
	31mm glass coverslips	NC1491415	Fisher Scientific	Used as side windows for sample chamber
Motion control	nPFocus1000 piezo stage	3715250	nPoint	1000 µm travel
	Controller LC.400	200761	nPoint	1 Axis
	LS-50 linear stage; 16 TPI (z-stage)	LS-50-AMERL	Applied Scientific Instrumentation	LS-50 3D stage stack-up
	Linear encoder option for z-drive (to attaining resolutions down to 50 nm)	LE-Z	Applied Scientific Instrumentation	
	LS-50 linear stage; 4 TPI (x- and y-stages)	LS-50-BMERL	Applied Scientific Instrumentation	
	Mount/bracket for linear stages	LS-5013	Applied Scientific Instrumentation	
	Plate for attaching linear stage to breadboard	LS-5012	Applied Scientific Instrumentation	
	Tiger controller, with xyz cards and joysticks	TG16_BASIC	Applied Scientific Instrumentation	
Instrument control	Supermicro motherboard with Intel C612 AHCI SATA controller	X10DRH-CT	CDW-G	See (1) below for specifications
	NI PXIe-1073 Integrated MXIe, 5 peripheral slots, PCIe-8361, 3 m cable	781161-01	National Instruments	
	Power cord, AC, U.S., 120 VAC, 2.3 m	763000-01	National Instruments	

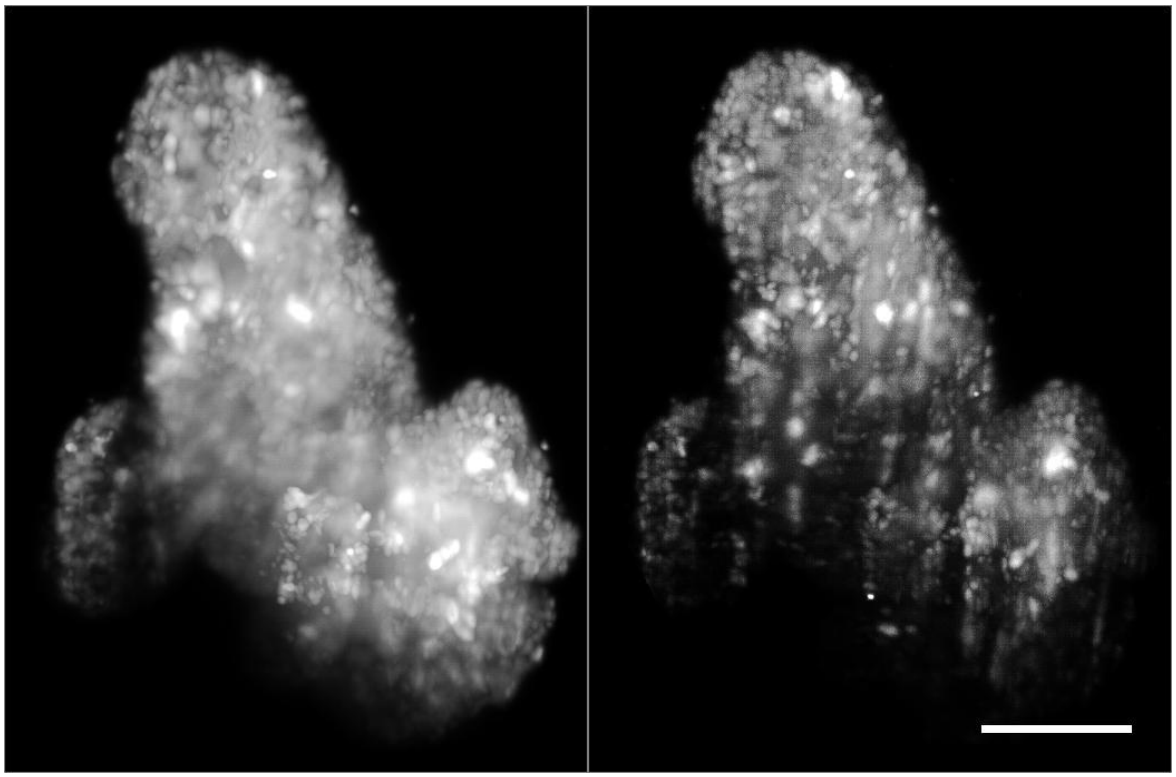
Subsystem/ module	Component	Catalogue number	Vendor	Notes
	NI PXIe-6363, X Series DAQ (32 AI, 48 DIO, 4 AO)	781056-01	National Instruments	
	CB-68LPR I/O connector block	777145-02	National Instruments	
	SHC68-68-EPM shielded cable, 68-D-type to 68 VHDCI offset, 2 m	192061-02	National Instruments	
	Mainframe with RS-232 computer interface	SIM900	Stanford Research	
	Scaling amplifier	SIM983	Stanford Research	
	PicoScope 2000 Series oscilloscope	2207B	Pico Technologies	2 channel; 70MHz
	BNC breakout box	PR35B32CMB	L-com	
Auxiliary	Monochrome CMOS camera	DCC3240M	Thorlabs	Behavior camera
	USB 3.0 I/O Cable	CAB-DCU-T3	Thorlabs	Cable for triggering
	35-50 mm fixed focal length camera lens	MVL50M23	Thorlabs	
	T-Cube LED Driver with Trigger Mode	LEDD1B	Thorlabs	Far-red LED
	780 nm, 200 mW mounted LED, 800 mA	M780L3	Thorlabs	
	1” diameter longpass filter	FEL0750	Thorlabs	Cut-on wavelength: 750 nm

(1): Windows 7 Professional w/SP1 64-Bit
1× SMC SuperChassis
CSE-836BE1C-R1K03B
2× Intel Xeon E5-2650 V4 CPUs, 2.2GHz, 12-Core, 30M Cache, 105W
4× 32GB DDR4 2400MHz ECC Registered DIMMs (128GB Installed)
2× 10GbE NIC Ports - Intel X540 dual-port LAN, RJ45 (onboard)
1× Integrated IPMI 2.0 with dedicated LAN
1× MSI 2GB GDDR5 64-Bit
GPU/Videocard, N730K-2GD5LP/OC
8× Empty 3.5"" Drive Bays with trays
1× H/W RAID Controller, LSI 3108, 2GB DDR3 Cache (S3108L-H8IR-16DD onboard)
Supported RAID Levels 0, 1, 5, 6, 10, 50, 60
2× 1000W Redundant Hot-Swap Power
1× Hot-Swap 512GB SATA 6Gb/s OS SSD drive, 2.5"", Samsung 850 Pro
8× Hot-Swap 4TB SATA 6Gb/s data drives, 7200 RPM, Seagate ST4000NM0035
1× DVDRW Slim Black SATA Samsung, SN-208FB/BEBE LG - #GTC0N

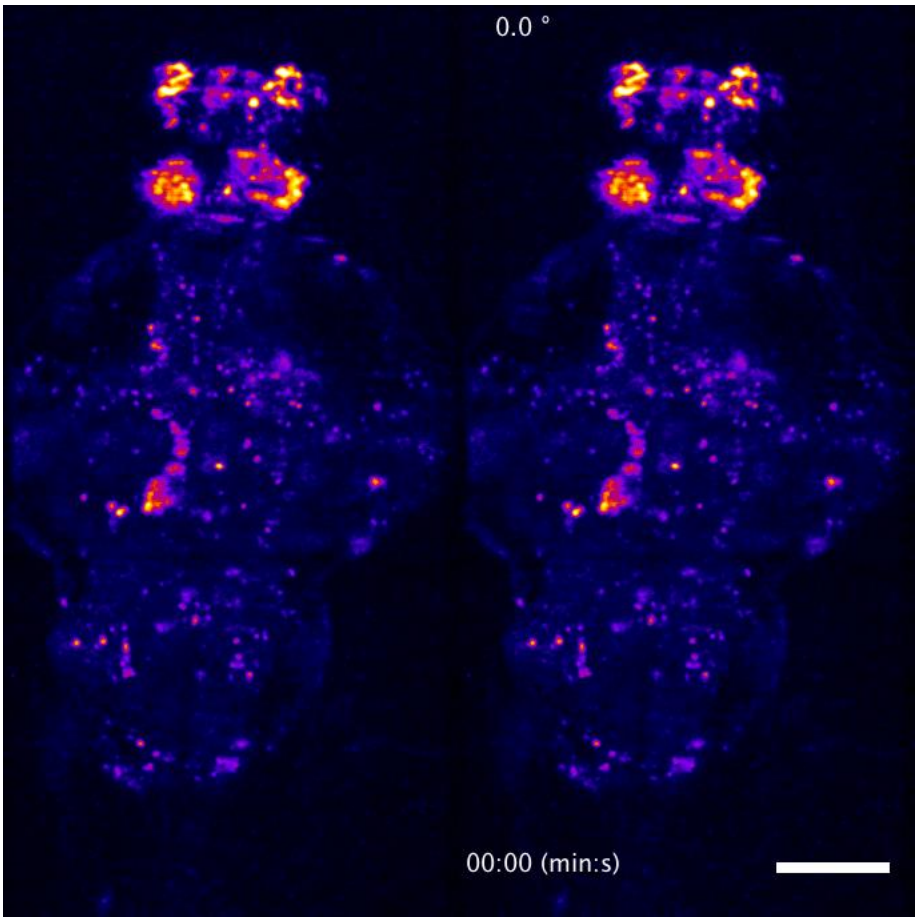
MOVIE CAPTIONS:



Movie S1. Light-sheet imaging of the dynamic motion of the beating heart of a 5-dpf transgenic larval zebrafish. Same dataset as presented in Fig. 7. Frames were captured at 85 Hz. Scale bar, 50 μm.



Movie S2. Volume rendering of fixed patient-derived tumor organoids expressing H2B-GFP, comparing one-photon (left) and two-photon recordings (right). Volumes are rotated around the *y* and *x* axes. Same datasets as presented in Fig. 8. Scale bar, 100 µm.



Movie S3. Dorsoventral (left) and rotating (right) maximum-intensity projections of a time-lapse recording of the whole-brain of the a 7-dpf transgenic larval zebrafish. Same dataset as presented in Fig. 9. Two-photon whole-brain functional light-sheet imaging was performed at 0.5 Hz. The video loops a 5-minute recording of large-scale neural activity in the behaving animal. Scale bar, 100 μm.

SUPPLEMENTARY NOTE 1

Simulations of scan lens performance using an achromatic doublet

We chose an achromatic doublet lens as a scan lens, instead of using specially-designed scan lenses which are more expensive. This decision was based on computer-aided optical modeling (Zemax, Radiant Zemax LLC) of commercially available lenses, showing that the achromatic doublet lenses have sufficient performance for our needs. We considered $1/e^2$ Gaussian beam diameters ≥ 1.5 mm and ≤ 4 mm; excitation wavelengths corresponding to 445 nm, 448 nm, 561 nm, 647 nm, 910 nm, and 1040 nm; and a maximum scan angle of ± 4°, which is equivalent to sweeping out a 2.56 mm^2 illuminated plane at the sample (given the illumination tube lens and excitation objective described above)—far exceeding the desired 0.5 × 1 mm^2 illuminated field. Being ultra-conservative about the maximum field angle ensured minimal aberrations as well as the flexibility to illuminate even larger areas (> 0.5 × 1 mm^2), if desired. The chosen achromatic doublet does a surprisingly good job of broadband (445-1040 nm) diffraction-limited performance over a large field angle, as illustrated in Fig. S1.

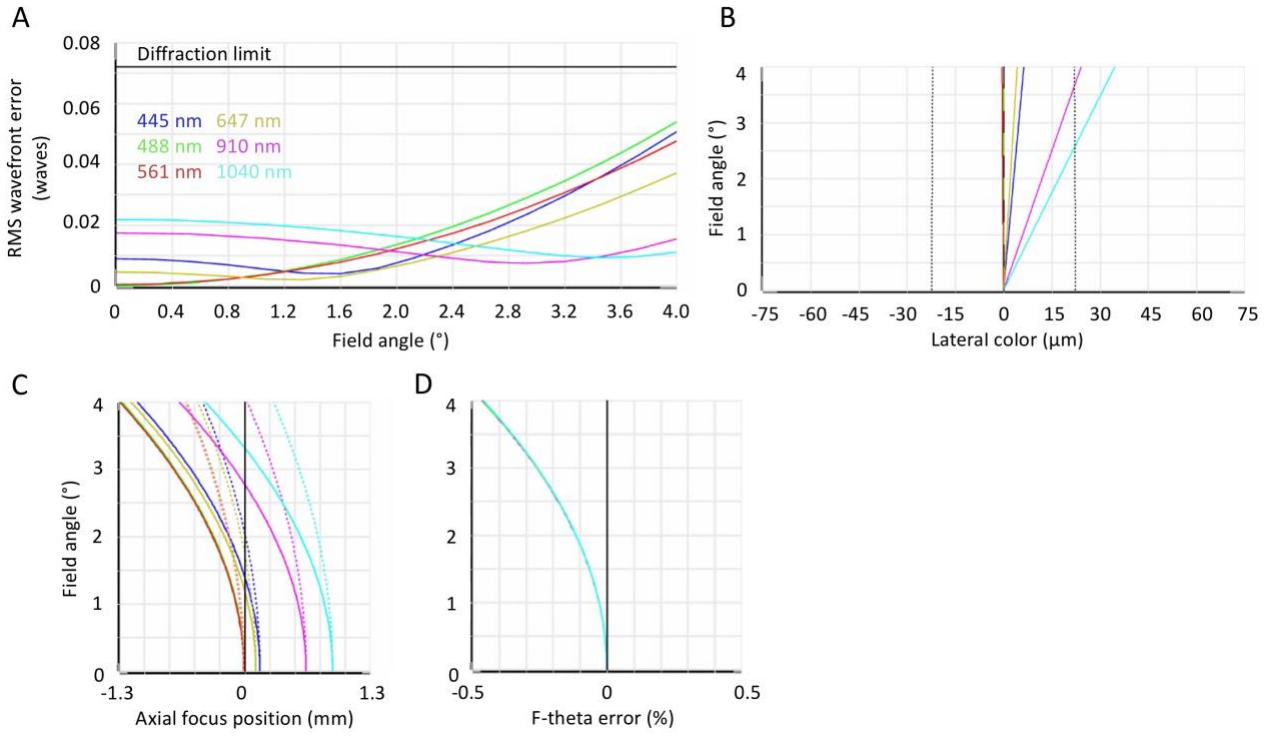


FIG. S1. Scan lens simulations of the chosen achromatic doublet (f = 150 mm, 50 mm diameter, VIS-NIR 49-391, Edmund Optics) using a $1/e^2$ beam diameter of 4 mm. (A) Transmitted wavefronts [measured by root mean squared (RMS) wavefront error] remain diffraction-limited, well beyond the desired scan range (for reference, ± 2° is equivalent to a 1.25 mm^2 illuminated field at the sample). (B) Using a 1000 nm Airy disc as reference, lateral color remains diffraction-limited for the desired ± 2° scan range; for scan angles > 2.5°, chromatic aberrations occur at 910 nm and 1040 nm. (C) Field curvature for the tangential and sagittal beam components, respectively represented by the solid and dashed lines. At the maximum scan angle of ± 4°, the field curvature is 1.3 mm for the tangential and 0.52 mm for the sagittal components; at the sample, this curvature translates to ~ 0.9 μm for the tangential and ~ 0.4 μm for the sagittal components, both of which are minor deviations relative to the illuminated field. (D) F-theta distortions are minimal: < 0.5% at the largest scan angle.