Automated High-Throughput Light-Sheet Fluorescence Microscopy of Larval Zebrafish

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Supplementary Information

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Supplemental Figure Captions

Supplemental Figure 1. Sample brightfield images of four larval zebrafish, captured and saved prior to light sheet fluorescence imaging. Notably, the orientation is random in the capillary, with a tendancy toward either "gut down" or "gut up." Scale bar: 100 μm.

Supplemental Figure 2. The number of neutrophils in 67 larval zebrafish, assessed from two-dimensional maximum intensity projections and from the full three-dimensional light sheet fluorescence scans. The former is 0.76 ± 0.02 of the latter, indicating that three-dimensional imaging is necessary to capture all the cells in a three-dimensional volume.

Supplemental Movie Captions

Supplemental Movie 1. Video of the automated, high-throughput light sheet microscope in operation. After a larval zebrafish, flowing through tubing, is detected and positioned, 3D scans in two colors in two different regions, are obtained. The final image (0:57-1:03) is of tdTomato-labeled bacteria and GFP-labeled neutrophils.

Supplemental Movie 2. A light sheet fluorescence scan through the anterior intestine of a larval zebrafish with GFP-expressing neutrophils evident as very bright objects above autofluorescent background. The total image depth is 475 μm (2.5 μm/slice x 190 slices). The maximum intensity projection of this scan is shown in Figure 2E.

Supplemental Table: Parts List

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Label in	Part and Description	Supplier / Manufacturer	Part no.
Excitation Optics			
1	Lasers: 458nm, 488nm, 561nm, 594nm; 20 mW	Coherent	Saphire
	Fiber launch	Thorlabs base + custom objective mount + Siskiyou fiber mount	
	Objective lens for fiber launch	Zeiss	5x plano-apochromat
2	Acousto-optic tunable filter (AOTF)	AA Opto-electronic	AOTFnC-VIS-TN 1001 / 340697
	Fiber switch (fiber = Nufern S460 HP)	Leoni	eol 1x4
3	Cage Mount for galvanometer mirror, 30mm	Thorlabs	GCM001
3	Galvanometer mirror	Cambridge Technology	6210H
4	PrePrism-sample chamber objective	Mitutoyo Plan APO 5X	Z03154214
Hardware related to the sample chamber			
	Sample chamber: PA11 Black plastic, 3D printed	Protolabs	3D printed
	Alternate sample chamber (blue): E-Shell 3000 biocompatible plastic	Envisiontec.com	3D printed
	Objective lens: Olympus 20x/0.5 Water Immersion Obj.	Edmund Optics	34556
	O-rings,various	McMaster-Carr	various
	Glass window, 20mm diameter, 2mm thick, VIS-NIR fused silica	Edmund Optics	49-642
	Heater: flex heater, sheet of ~10	Omega.com	KH-kit-efh-15001
5	Prism, 20mm, N-BK-7 rt angle prism	Edmund Optics	32-334
5	Prism mount: mini-translation stage, one axis, linear	ThorLabs	DT12
5	Prism holder	UO	custom
5	45° mount	Edmund Optics	59001
5	1.5" straight mirror mount	Edmund Optics	33497
5	1.0" straight mirror mount, mount only	Edmund Optics	33-501
	English long travel rack & pinion, camera adjustment	Edmund Optics	59-333
	One knob stage	Edmund Optics	59-331
	Rail, 66mm square	ThorLabs	XT66
	Rail, Dovetail	ThorLabs	XT66SP
	Rail carriages	ThorLabs	XT66P2
	6mm rods for cage system/sample chamber holder	ThorLabs	ER-xx, various lengths
Fluidics			
	Flow cell and holder		
11c	Flow cell: 3/16" dia. Clear acrylic rod	McMaster-Carr	UO Machine shop
11b	Flow cell: 1/2" blunt stainless steel needle 18gauge	McMaster-Carr	75165A675
11d	Flow cell: Round glass capillary, 50mm, 0.7mm ID, 0.87mm ID	Vitrocom Technical Glass	cv7087-B
	Flow cell: Square glass capillary, 50mm, 0.7mm ID, 0.140mm Wall	Vitrocom Technical Glass	8270-050
	Flow cell: Tub and Tile Silicon caulk	Hardware store	
11a	Tubing, "Silastic," 0.74mm ID, Platinum cured	Fisher Scientific	11-189-15C
	Luer lock plastic valve		
9	Syringe Pump, (BenchTop Style) 1/10 ml glass syringe	KD scientific	111
	Metal Y tube, 0.05" 0.033" 1/4"	ztubes.com	HSCY-18
	Relay (for valves), 60 VDC/3.5A, 5Volt Logic	Grayhill	70G-0DC5x16
10	12V solenoid pinch valves (x6) 15PSI PN98302	Cole Parmer	
10	N.O. Pinch Valve, 0.75" dia., 0.030" ID-0.065" OD Tubing, 12 VDC	Clippard	N PV1-1O-01-122

10	N.C. Pinch Valve, 0.75" dia., 0.030" ID-0.065" OD Tubing, 12 VDC	Clippard	NPV1-1C-01-122
10	N.C. Pinch Valve, 15psi 12V	ColePalmer	98302-02
Other Hardware			
8	Hamamatsu Orca Flash 4.0 Camera	Hamamatsu	C11440
	Firebird CL 2011 camera link board	Active Silicon	AS-FBD-1XCLD-2PE8
6	LED light (Bright field illumination)	ThorLabs	MCWHL5
	LED driver	ThorLabs	LEDD1B M00400850
	Stage controller	Applied Scientific Instrumentation	TG-1000-16, TG16-XY:5A-Z:5U
	xyz stage	Applied Scientific Instrumentation	LS-5507,+?
	xyz arm, 8020, 1" x ~12" long aluminum channel	McMaster-Carr	47065T101
	Data Acquisition (DAQ) Board	National Instruments	USB-6343 X-eries