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OpenSPIM: an open-access light-sheet microscopy platform

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To the Editor:

Light-sheet microscopy is revolutionizing biology by enabling live *in toto* imaging of entire embryos or organs with minimal phototoxicity¹. We present an open hardware and software platform for constructing a customizable microscope for selective-plane illumination microscopy (SPIM). The OpenSPIM platform is shared with the scientific community through a public website (<http://openspim.org/>), making light-sheet microscopy more accessible so that it can be optimized for various applications.

SPIM is ideally suited for capturing anatomy and patterns of gene activity in large developing specimens expressing fluorescent reporters². However, because genomes contain thousands of genes and development can last for days, it is necessary to increase the throughput of SPIM acquisitions. We propose to do this by building arrays of affordable SPIM systems that allow the parallel imaging of many samples.

The initial version of the OpenSPIM setup implements a single-sided illumination and single-sided detection light-sheet arrangement (Fig. 1a and Supplementary Fig. 1), the most basic realization of the SPIM principle³. The microscope is controlled via a dedicated plugin (Supplementary Fig. 2) extending the popular open-source microscopy control software μ Manager⁴ running inside Fiji⁵ on standard computer hardware. This architecture enables active feedback of on-the-fly image processing on image acquisition and facilitates reconstruction and analysis⁶ (Fig. 1b). To demonstrate the ability of OpenSPIM to image large specimens, we imaged a zebrafish embryo expressing histone H2A–EGFP by acquiring six overlapping fields of view (Fig. 1c and Supplementary Video 1). OpenSPIM can capture fast biological processes, such as the beating zebrafish heart, by recording a single plane at the maximum frame rate of the camera (Supplementary Video 2). For samples that strongly scatter light, such as *Drosophila melanogaster* embryos, OpenSPIM

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offers multiview acquisition (Fig. 1d,e and Supplementary Videos 3 and 4)—which, in combination with Fiji's multiview registration⁶, can be used to record embryonic development (Supplementary Fig. 3 and Supplementary Video 5). Finally, the four-dimensional recording of the dynamics of gene expression pattern formation during development (Fig. 1f and Supplementary Video 6) is the representative application for which the parallelization offered by OpenSPIM provides the necessary imaging throughput.

The unique feature of the OpenSPIM project is its emphasis on an open development process and community. We have established a wiki site that documents in great detail the building, alignment, software and operation of OpenSPIM. The wiki contains a comprehensive list of parts (http://openspim.org/Table_of_parts); detailed instructions for assembly supplemented with photographs, drawings and videos (http://openspim.org/Step_by_step_assembly); narrated tutorials for light-sheet alignment and sample preparation (<http://openspim.org/Operation>); and descriptions of the open-source software that are useful for users as well as developers (<http://openspim.org/Category:Software>).

The OpenSPIM system was intentionally designed to be simple, compact, modular and accessible. The current configuration of OpenSPIM (Supplementary Fig. 4a) can be extended to more advanced SPIM geometries (two-sided illumination and/or detection) (Supplementary Fig. 4b,c), and its modular properties will allow users to multiplex time-lapse acquisitions by building several setups operating in parallel (OpenSPIM farm; Supplementary Fig. 4d). Its simplicity makes it an excellent teaching tool for both applying and building SPIM technology. The combination of open-access hardware and open-source software creates a platform adapt-able for specific imaging tasks and a starting point for prototyping of new concepts. The OpenSPIM wiki invites interested researchers to build the setup and in turn contribute their knowledge and innovations back to the wiki site. We envision that this will nucleate an interdisciplinary community in which biologists interested in using SPIM will interact with technology developers and bring to life innovative ideas for addressing complex scientific questions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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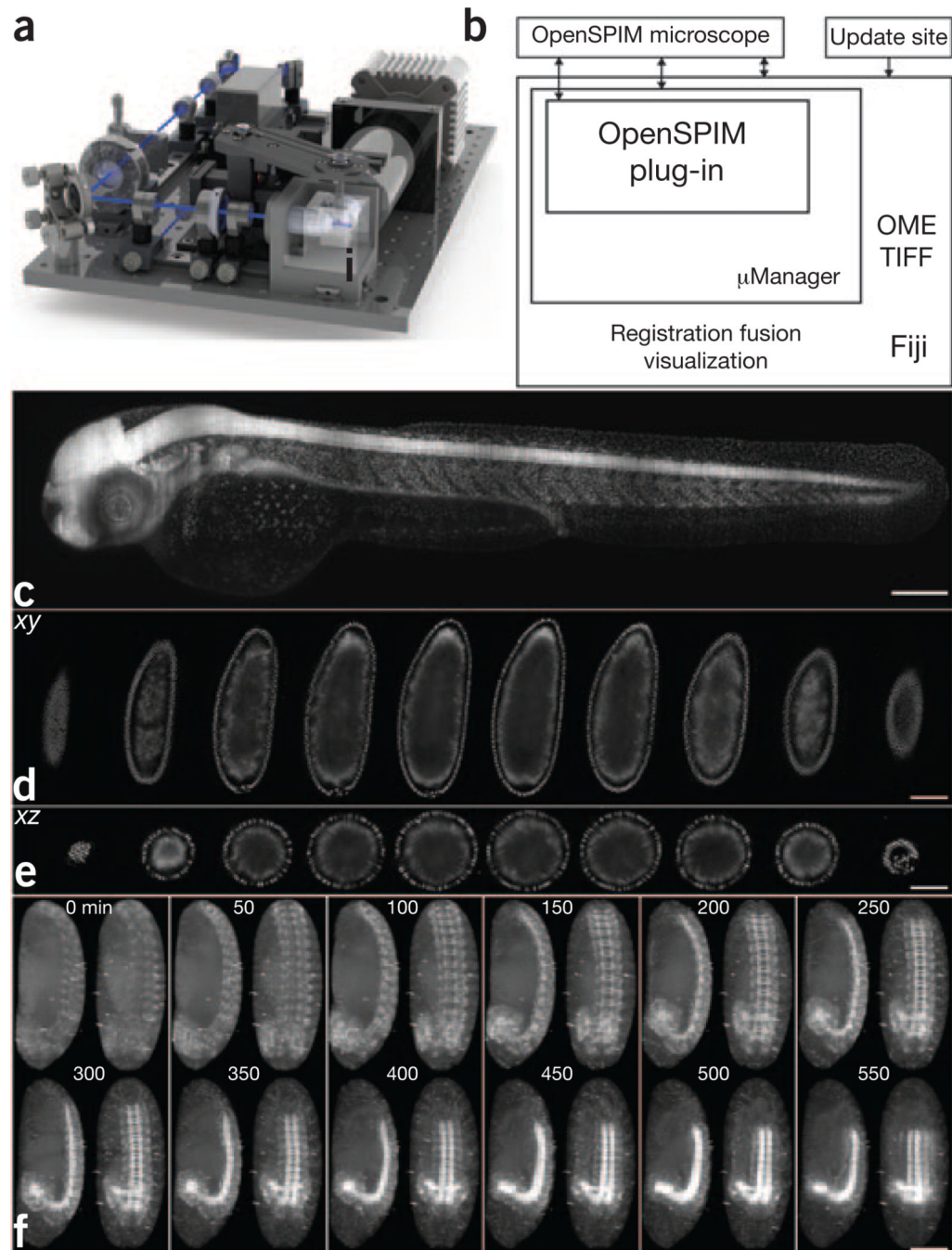


Figure 1 | OpenSPIM hardware, software and data.

(a) SolidWorks rendering of the OpenSPIM setup. (b) Schematic of the OpenSPIM steering software architecture. (c) A 2-d-old zebrafish larva expressing H2A-EGFP under the control of β -actin promoter Tg(Bactin:H2A-EGFP) was imaged as a set of six overlapping fields of view. (d,e) Lateral (d) and axial (e) planes through a blastoderm-stage *Drosophila* embryo expressing histone-YFP imaged from six views at 6- μ m steps of the light sheet. The data were reconstructed using a bead-based registration algorithm and fused with multiview deconvolution in Fiji. (f) *Drosophila* embryos expressing Csp-sGFP protein fusion under

native promoter control imaged from five views every 10 min. Maximum-intensity projections along the lateral and dorsal-ventral axes are shown for every fifth time point, highlighting the dynamic morphogenetic movement of the nervous system. Scale bars, 100 μm .

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