

sequence (**Supplementary Note 1** and **Supplementary Fig. 3**). The analysis highlighted an important role of chemokine signaling in colorectal cancer progression and revealed previously undiscovered dynamic gene-expression patterns that may be useful for future therapeutic interventions based on anti-inflammatory strategies. Our second example integrated somatic mutation and copy number–variation results from The Cancer Genome Atlas glioblastoma multiforme (GBM) study<sup>3</sup>. The analysis identified known networks involved in GBM and presented a novel FN1-centered network that may provide insights into both tumor progression mechanisms and clinical management of GBM (**Supplementary Note 1** and **Supplementary Fig. 4**). Moreover, by visualizing somatic mutation data from individual samples together with network-module information, the analysis revealed a mutually exclusive mutation pattern for genes in the PIK3R1-centered network (**Supplementary Note 1** and **Supplementary Fig. 4I**).

In contrast with typical network visualization tools, NetGestalt allows multiscale representation and navigation of the data, and it adjusts graphical presentations to the level of detail appropriate to a particular scale. It also allows simultaneous visualization of different types of data within the same framework to facilitate data integration. Moreover, NetGestalt complements genome browsers<sup>4</sup> in visualizing the functional relationship of genes located on different chromosome locations. This facilitates, for example, the discovery of pathways and networks that link important genomic alterations (**Supplementary Fig. 4p**). A more detailed comparison between NetGestalt and other related tools is provided in **Supplementary Table 4**. Data sets used in this study (**Supplementary Table 5**) can be explored through the NetGestalt website, and a user manual is available in **Supplementary Note 2**.

Note: Supplementary information is available in the online version of the paper (doi:10.1038/nmeth.2517).

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#### AUTHOR CONTRIBUTIONS

B.Z. led the project and oversaw the software development and data analysis. Z.S. designed the software architecture and implemented the software. J.W. prepared the data, developed the NetSAM package and performed the bioinformatics analysis. B.Z., J.W. and Z.S. wrote the manuscript.

#### COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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## OpenSPIM: an open-access light-sheet microscopy platform

**To the Editor:** Light-sheet microscopy is revolutionizing biology by enabling live *in toto* imaging of entire embryos or organs with minimal phototoxicity<sup>1</sup>. 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.

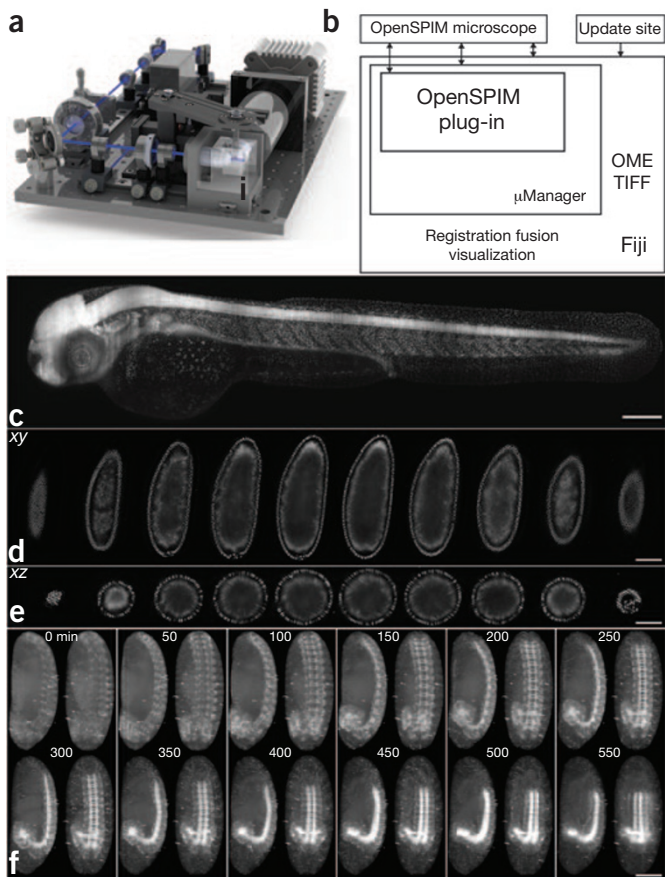
SPIM is ideally suited for capturing anatomy and patterns of gene activity in large developing specimens expressing fluorescent reporters<sup>2</sup>. However, because genomes contain thousands of genes and development can last for days, it is necessary to increase the throughput of SPIM acquisitions. 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 principle<sup>3</sup>. The microscope is controlled via a dedicated plug-in (**Supplementary Fig. 2**) extending the popular open-source microscopy control software  $\mu$ Manager<sup>4</sup> running inside Fiji<sup>5</sup> on standard computer hardware. This architecture enables active feedback of on-the-fly image processing on image acquisition and facilitates reconstruction and analysis<sup>6</sup> (**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 offers multiview acquisition (**Fig. 1d,e** and **Supplementary Videos 3** and **4**)—which, in combination with Fiji's multiview registration<sup>6</sup>, 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](http://openspim.org/Table_of_parts)); detailed instructions for assembly supplemented with photographs, drawings and videos ([http://openspim.org/Step\\_by\\_step\\_assembly](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



**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  $\beta$ -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- $\mu$ 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  $\mu$ m.

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 adaptable 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.

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## OpenSpinMicroscopy: an open-source integrated microscopy platform

**To the Editor:** Light-sheet microscopy has recently emerged as the technique of choice for fast, high-resolution three-dimensional (3D) imaging of whole organisms, one that also features low photo-damage<sup>1,2</sup>. Unlike confocal or multiphoton systems, light sheet-based microscopes can easily provide a combination of multiple views by rotating the sample. Multiview imaging solves the problems caused by light scattering, shadowing and anisotropy that are inherent to uniaxial optics. Sample rotation is also a fundamental aspect of optical tomography techniques<sup>3</sup>. However, image-acquisition and microscope-control software is not, by default, prepared to handle stage rotation.

We present an open-source, fully customizable platform for the control of light-sheet microscopes and optical projection tomography (OPT) setups operating in different modes, based on Arduino and  $\mu$ Manager<sup>4</sup> hardware. Through our web page (<http://uic.igc.gulbenkian.pt/micro-dslm.htm>), we provide full access to hardware (sketches, a parts list and circuit designs) and software (plug-in code,  $\mu$ Manager device adaptors and Arduino firmware). This integrated platform allows the implementation of a vast array of imaging approaches and offers the versatility to image samples ranging from small organisms to whole embryos (~1–2 mm) and larger samples up to 1–2 cm. The OpenSpinMicroscopy plug-in facilitates handling of sample rotation and multiview 3D data sets, fully controlling either selective-plane illumination microscopy (SPiM), digital scanned