Microscopy: OpenSPIM 2.0

Vivien Marx

A maturing open hardware and open-source software movement seeks to expand DIY light-sheet microscopy.

After taking a short course, he's getting ready to build a light-sheet microscope with colleagues in the imaging facility, says Florian Vollrath, a physicist and programmer in the imaging facility at the Max Planck Institute (MPI) for Brain Research in Frankfurt. They want to image cleared brain samples as large as 10 cm^3. With light-sheet microscopes, researchers section samples with a slice of light. There are commercial instruments and build-your-own models.

"I like the idea of having an open-source community for a light-sheet system," says Vollrath. He will be using information offered on a website that is part of the open hardware and software movement and devoted to open selective plane illumination microscopy (OpenSPIM)[1]. Even people with little or no experience building optical setups need only a bit of assistance to get a system up and running, he says.

Johannes Girstmair, a biology PhD student at University College London in the lab of Maximilian Telford, assembled, built and configured an OpenSPIM instrument from scratch and without any previous experience[2]. He is using it to address evo-devo questions, such as by studying the flatworm *Maritigrella crozieri*'s spiral cleavage pattern and its free-swimming planktotophic larval stage. Girstmair first tried out OpenSPIM when he visited Pavel Tomancak's lab at the Max Planck Institute (MPI) for Molecular Cell Biology and Genetics in Dresden. Girstmair and his colleagues imaged green-fluorescent-protein-tagged nuclei and membranes and they generated detailed 3D reconstructions of *Maritigrella* embryos and larvae, which have been difficult to image. "At this point we could really see the potential of OpenSPIM," says Girstmair.

Johanna Gassler, a PhD student in the lab of Kikue Tachibana-Konwalski at the Institute of Molecular Biotechnology in Vienna, isn't yet building her own microscope but knows a few biologists who are glad they found the courage to do so. Building one's own microscope, she says, allows you to think about what is needed for a sample and experiment and to tailor everything to those needs.

V. Surendranath

OpenSPIM lets researchers take their microscopes on the road.

A wide range of OpenSPIM instruments have been built: eduSPIM is a pared-down model used to teach microscopy to science museum visitors in Dresden[3]. Other models are complex and barely resemble the basic setup, says Tomancak. Others still are modifications, such as Girstmair's T-openSPIM, which forms a light-sheet with dual-sided as opposed to the more typical one-sided illumination.

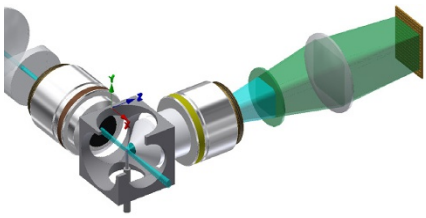
Back in 2010, over lunch with his MPI colleagues, Tomancak launched OpenSPIM, and he set up online resources with a parts list and assembly videos to get the instrument into the hands of as many biologists as possible. Labs had begun building their own setups when light-sheet systems were not yet commercially available. That led to a groundswell of activity motivating Tomancak and others to help labs trouble-shoot snafus at their optical tables and help biologists with their experiments. In the works now, says Tomancak, is OpenSPIM 2.0.

If funding comes though, OpenSPIM 2.0 will capture images faster and will be a more modular system that lets labs assemble many types of microscopes, says Tomancak. One lab might need a particular configuration for an optogenetics experiment. Some labs need to capture ten image stacks per second, whereas others do not need such imaging speed but prioritize stability enabling them to image precious samples for five days straight. He himself would like to build a higher-throughput OpenSPIM system to image gene expression patterns of thousands of genes. OpenSPIM 2.0 will address these disparate needs and maintain affordability and commitment to community and open access principles, he says. The new system is under development, making this a good time to take stock of some user experiences.

SPIM advantage

Selectively illuminating only the observed single focal plane reduces photo-damage to large samples or organisms imaged *in vivo*. That is one of several advantages that the SPIM inventors and developers, Ernst Stelzer and colleagues at the European Molecular Biology Laboratory (EMBL), highlighted when they applied the method to biology in 2004[4]. Experimenters can gaze from multiple angles at samples—such as the rather opaque *Drosophila* embryos—and can watch events unfold and capture 3D gene and protein expression patterns.

His OpenSPIM images are neither as crisp nor as high-resolution as the ones he can obtain with a confocal microscope, says Girstmair. But given the way specimens are mounted on a confocal, he can't readily capture images from multiple angles. And with a difficult specimen that is opaque and yolk-filled, "it's like 3D-reconstructing the moon,"



E. Stelzer, Goethe University

Labs can section samples with a slice of light when using light-sheet microscopes.

he says. To see the 'dark side', an experimenter has to rotate the sample and re-capture images. Observing a developing embryo with confocal imaging would also mean exposing the embryo to the full laser beam during its development; the first cleavage in *Maritigrella* can take up to two hours. As he sets out, he appreciates that his OpenSPIM setup illuminates a single plane and not the whole embryo, is fast and acquires many more images in a shorter time frame than with a confocal. This matters because phototoxicity is best not underestimated with the polyclad flatworm, says Girstmair.

With his fixed samples, Tiago Pinheiro, a PhD student at Karolinska Institute, wants to tackle a time crunch with light-sheet microscopy. He works on cleared salamander brains to study regeneration of dopamine neurons. This animal's brain is around 25 times smaller than a mouse brain, but just to image the salamander's olfactory bulb he had to leave a sample on a confocal for 8–12 hours. Studying neurite projections would have taken even longer and bleached the sample.

As Pinheiro starts out with light-sheet microscopy, he likes that it lets him look at an entire volume and gain perspective on how cells and fibers relate to one another so he can extract many quantitative parameters. With OpenSPIM, he hopes to ramp up the scale at which he works. Harvesting data in at least three replicates at each time point and for each group of animals—even in a small experiment with two time points, a control and a lesion group—means imaging 12 brains. Light-sheet microscopes are fast, but given that the lab's commercial systems are heavily used, he would not be able to book the imaging time he would need. With OpenSPIM scientists can see whether their scientific question can be broached with light-sheet microscopy. If yes, says Pinheiro, building one or even several such microscopes could speed up image acquisition. OpenSPIM instruments are also visual teachers. "I gained a very in-depth understanding on how light-sheet technology works by seeing the 'naked' microscope," he says, which is not possible with commercial systems.

After he saw the Stelzer paper, Tomancak, then a postdoctoral fellow in Gerald Rubin's lab at the University of California, Berkeley, ran into his PI's office saying, as he recalls, "This is the future, we have to use it." At the time, Rubin was not quite convinced, says Tomancak, but in a recent conversation between the two men, Rubin, now director of the Janelia Farm Research Campus, told Tomancak his excitement back then was justified. Tomancak now co-organizes an annual European Molecular Biology Organization (EMBO) course on OpenSPIM and light-sheet microscopy.

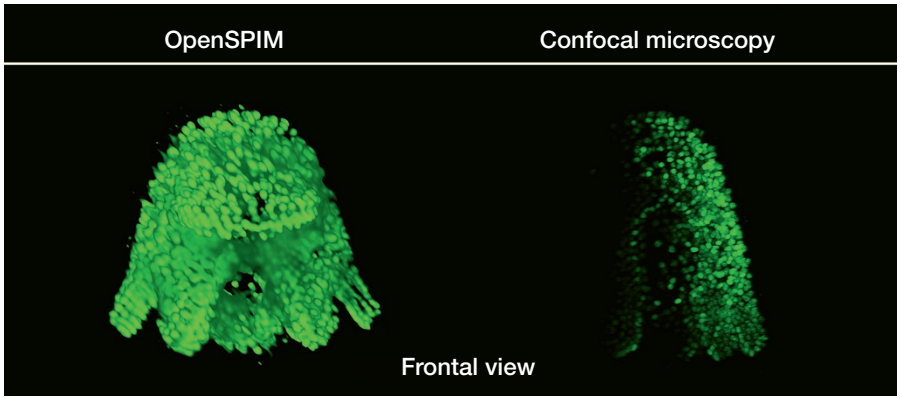
Early OpenSPIM adopters were optical technology developers, says Tomancak, but developmental biologists quickly realized OpenSPIM would let them see an entire developing system, potentially monitor every cell and study morphogenesis as it unfolded. Researchers in neurobiology, physiology, tissue culture and cell biology have been catching on and might be next, he says.

One developmental biologist contacted by *Nature Methods* who did not wish to be identified said that he has worked only with early OpenSPIM models. "From what I have seen of the homemade versions, they tend to be finicky and usually can only be operated by the builders," he says. The systems were not suited for general use and were not user-friendly. "That said, things may well have improved now," he says.

Marianne Bronner, a developmental biologist at California Institute of Technology, has used both commercial and home-built SPIMs and sees merit in both. The commercial light-sheet microscopes perform well for her purposes and, she says, "I do think that a specialized home-built one is more likely to be better resolution for the purpose, since it's designed with a single use in mind."

Bronner works regularly with Andres Collazo, who directs Caltech's biological imaging facility, where there are two commercial light-sheet systems. As part of a class he teaches, he and four students built an OpenSPIM instrument. They got "just a little help from me," says Collazo. Although the OpenSPIM system is quite good, he says, it has only one color laser, which makes it suited only for specific applications. The light-sheet is created with a cylindrical lens, which in his view is not as good as scanning a laser in order to create a light-sheet that also provides more illumination. Building the scanner would, however, raise the price tag on the system that cost him around $18,000, excluding the camera. Commercial systems are more powerful and flexible, but they cost between $200,000 and $500,000 depending on the system. "Given all that I am quite a fan of OpenSPIM and think it would be very useful for very specific applications," he says. Collazo's OpenSPIM was based on OpenSPIM 1.0 and, he says, "I'd be interested in what they do with 2.0."

Some of the troubleshooting of his OpenSPIM system involved software-based questions, and "we never quite got the camera focal plane to line up with the specimen focal plane," says Collazo. "It was only a little off but you could tell." He collaborates with the Caltech chemistry department's machine shop, where colleagues made the parts he needed according to the OpenSPIM website and readjusted them, too. The OpenSPIM setup does not have an eyepiece, which can take some getting used to, but he is used to looking at specimens through a camera, says Collazo. "The worst



J. Girstmair

Johannes Girstmair built an OpenSPIM configuration to study evo-devo questions using, for example, the polyclad flatworm *Maritigrella crozieri*.

Katrin Boes

"This is the future, we have to use it." That's what Pavel Tomancak recalls saying when he first heard about light-sheet microscopy.

thing is when the software says it's a live image but it actually locked up," he says. "You can mess up the alignment fast that way."

Building an OpenSPIM was fun, says Girstmair, once all the pieces were collected and laid out. Some of the parts have to be machined, such as acquisition chambers and axis holders, he says. He says it would help to have a catalog from which to order the parts best suited to a given system. "The fun usually ends when it comes to the alignment of the lasers, at least in the beginning," he says. But even that became easier as he grew more familiar with moving mirrors and placing the beam splitter at the right spot.

Tomancak acknowledges that labs might need help with aligning their light-sheet. They also need to know their system, such as the thickness of their light-sheet. Two OpenSPIMs built in different labs can have light-sheets of differing thickness, which may be due to alignment or optics decisions, he says. Such parameters matter with commercial systems, too. "Light-sheet is called a sheet but it's not a sheet, essentially, it's like an hourglass, it's thin in the middle and becomes thicker progressively," he says. This aspect matters for specimen placement.

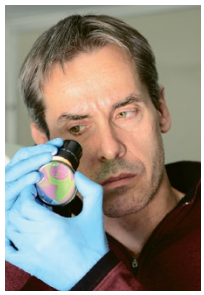
At Stelzer's laboratory at the Goethe University in Frankfurt, biologists are constantly using his commercial as well as his five home-built light-sheet-based fluorescence microscopes (LSFMs). They make sure that the specimen is well placed and observed along the optimal angle, he says, and they spend no time aligning these systems. "It is certainly a nice experience to build a light-sheet microscope but I always recommend to start with a conventional fluorescence microscope," he says, and to then compare performance between home-built and commercial systems.

With home-built SPIM instruments, stability is the real issue, says Stelzer. In his lab, users do not touch a single instrument screw. Unless the specimen moves, it remains in focus for several days of imaging, and the system remains stable. With stability in mind, he had his monolithic digital scanned light-sheet microscope milled from a single aluminum block[5]. In his experience with optical systems, neither mirrors nor lenses need 'many degrees of freedom' to provide optimal performance. With a diffraction-limited microscope scientists can calculate everything with micrometer-level precision. "Any large deviation between calculation and measurement indicates you have made a mistake," he says.

The two optical paths of illumination and detection in a light-sheet microscope should be regarded as an opportunity to do things differently and, eventually, better, says Stelzer. Any field-of-view issue can be addressed. For example, to image a spheroid that essentially fills the field of view, a researcher will want a fairly wide, and even axial, resolution. When imaging the primary root of a plant—a long, thin object in the center of the camera's field of view—a small field of view along the illumination axis suffices. And a scientist can reduce the light-sheet size by a factor of two or four. "I am seriously afraid that bad images and bad sample preparation give LSFM a bad reputation," he says.

Learning by building

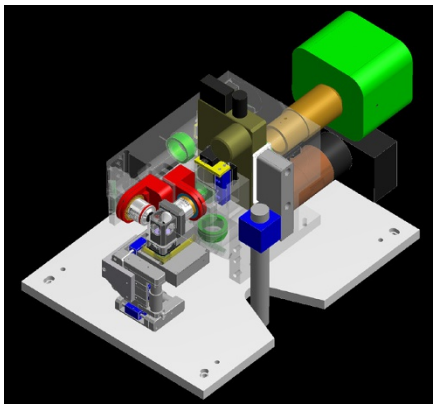
Jeff Carmichael, a technical product manager at Chroma Technology, which makes filters, generally likes the idea of OpenSPIM and calls OpenSPIM 1.0 a basic configuration. One omission in the parts list he sees is a laser 'clean-up' filter, which is an excitation filter with a narrow band pass that cleans up laser emission beyond a specified wavelength. There is, he says, a family of laser diodes with nanometer (nm) emission ranges needing clean-up: those in the 405-nm-ish, 440-nm-ish and 640-nm-ish ranges.



Chroma Technology

OpenSPIM builders likely need laser clean-up filters, says Jeff Carmichael.

Issues can crop up due to the angle of incidence of the light impinging on emission filters or dichroics. With interference filters, a 'blue-shift' can occur because of the way light at 'high' angles of incidence behaves as it passes through the filter's thin film stack of coating layers, says Carmichael. For example, a filter with a band pass from 520 nm to 550 nm might behave like a filter



E. Stelzer, Goethe University

Home-built SPIM instruments can be unstable, says Ernst Stelzer. He has built a digital scanned light-sheet microscope milled from an aluminum block.

with wavelengths from 517 to 549 nm. Ten degrees can be a problem and "20 degrees can be a killer," he says. This might not affect the actual fluorescence signal, but when the emission filter no longer blocks the excitation effectively, the image gets noisy, he says. There are ways to design interference filters in anticipation of a larger angle of incidence. Off-the-shelf filters can work, he says, but his company and others can make filters that specifically address this issue.

Sam Rubin, who manages imaging systems and life science activities at ThorLabs, is glad to see movements such as OpenSPIM. From the optical breadboard and rails to mounts to dielectric mirrors, many components in the OpenSPIM recommended parts list are ThorLabs products. The company has around 100 tech support engineers worldwide to advise labs that might need a component with certain dimensions. To help debug a problem, company engineers can re-create part of the customer's setup.

The company offers around 20,000 different components used by the life science and physics communities. Annually, 2,000–3,000 components are added, most of which are "customer-inspired," says Rubin. For example, an OpenSPIM researcher might have modified a mirror mount, an alteration that the company incorporates into a new product. One such customer-modified product is a microscope objective adaptor, which results from a customer seeking an adaptor for an "exotic" objective, he says. Objective sizes vary greatly; those with long working distances have many types of threads. Instead of charging the scientist non-recurring engineering costs to develop and make this part, ThorLabs made the adaptor for this researcher and for anyone else who wanted to buy it.

In recent years, ThorLabs started selling complete imaging systems such as multi-photon microscopes, but it does not yet offer systems or modules for light-sheet microscopes, only components such as those needed by the OpenSPIM community. All their imaging systems are open-hardware instruments. "We almost encourage our customers to hack it and to improve it," says Rubin. They post photos of hacked systems and integrate many hacks into their systems. The company also sells modules, such as aligned and calibrated optical systems for labs that do not wish to build these on their own from components.

As Rubin explains, ThorLabs is influenced by information-sharing movements such as the OpenSPIM community. A company's team of engineers cannot be better than a large crowd of developers. ThorLabs shares its hardware blueprints and offers imaging systems with open-source software. "I think that's a leaf out of the OpenSPIM book in a way," he says.

Maturing a movement

Tomancak acknowledges that the major hurdles people in the OpenSPIM community currently encounter are software issues. Without knowledge of computing and programming, they might need help from others. With more funding, he says, there will be more support for users.

After he built OpenSPIM 1.0, his funding levels dipped, but Tomancak has recently landed funding to advance the Fiji software package[6]. Because the microscope captures sample images at multiple angles, OpenSPIM images need to be registered, fused, deconvolved and downsampled to create usable data, he says. Fiji, developed in his lab, is an open-source software package for those purposes. Some of the developers have moved on to their own labs, but to improve the software and teach others how to use it he and colleagues at the Laboratory for Optical and Computational Instrumentation at the University of Wisconsin are organizing hackathons to bring together original developers and new researchers.

The researchers also built an interface to μManager, developed in Ron Vale's lab at the University of California at San Francisco and now maintained by developers at a company called Open Imaging. The OpenSPIM interface to μManager in Fiji lets users analyze images captured with their OpenSPIM instruments, and they can both process and analyze data from commercial instruments such as those by Leica and Zeiss, says Tomancak.

The OpenSPIM software works for him, says Girstmair, and he is also happy with μManager. "But if somebody would improve certain SPIM plugins and create a more robust, even more stable OpenSPIM software, that would be great to have," he says.

Building an OpenSPIM instrument does not just mean having a great tool all for yourself, says Girstmair; it usually also attracts the interest of many other researchers around you. That can help organize new collaborations, although there is also a danger of committing a lot of time to other people's projects. "OpenSPIM might not be perfect, or as easy to use as a commercial microscope, but what one can get out of it in terms of image quality keeps surprising me again and again," he says.

Overall, the OpenSPIM movement is "not dependent on us," says Tomancak. He is pushing to advance software and hardware and to support the OpenSPIM community. Stay tuned for OpenSPIM 2.0.

In Stelzer's view, researchers have yet to take full advantage of what LSFMs can already do. "The simplicity of the design allows us to build very basic instruments that ignore all ergonomic issues and simply concentrate on the specimen and the biological question by optimizing the preparation and taking full advantage of the performance of the light source and the sensors," he says. For the medium term, he believes that harnessing the spatiotemporal analysis of intensity fluctuations, such as with fluorescence correlation spectroscopy, and the fast modulation of the excitation light and the fast phase-shifted observation of the emitted light, such as in fluorescence lifetime imaging, will become standards in light-sheet fluorescence microscopy. That, he says, converts an LSFM into a biochemistry laboratory.

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