Microscopy made to order

The proliferation of versatile open software and hardware for microscopy is helping to democratize biological imaging for both current and aspiring scientists.

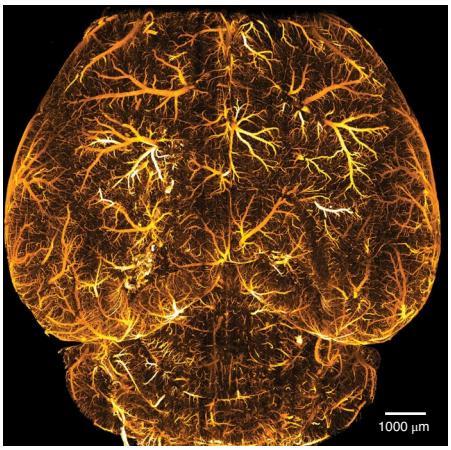
Michael Eisenstein

Manu Prakash's microscopes have gone on scientific expeditions that have historically been the domain of intrepid adventurers. "People have shipped Foldscope to outer space in little balloons," says Prakash, a researcher at Stanford University. "We have submerged Foldscopes deep in the water in lakes, and Foldscopes are used in tree canopies to do microscopy when there are no other instruments that you can bring there."

A simplified, paper-based instrument that can achieve micron-resolution biological imaging with a price tag of just a few dollars, Foldscope is one of the more extreme examples of a broader effort by microscopists to democratize their field. The 'open-hardware' movement is providing blueprints and documentation that researchers around the world can readily access to build and customize—or even 3D print from scratch—instruments capable of delivering research-quality data.

These projects share roots with the open-source software movement, which has a long history in the microscopy world. Open source is often reductively viewed as simply meaning 'free'—and such software packages typically are—but transparency and accessibility are also essential elements of any open-source initiative. Kevin Eliceiri of the University of Wisconsin at Madison, lead developer on the widely-used ImageJ software toolbox, notes that it is a mistake to think of these tools as just a cheap alternative to commercial products. "There are different flavors of open source," he says. "There's open source you do because you want to save money, or because you want to build and adapt something for your own lab—or because there's no alternatives." So, too, in the instrument world, where open-hardware projects run the gamut from the Foldscope to initiatives like MesoSPIM, which helps users construct cutting-edge light-sheet microscopes, with a whole spectrum of options in between.

This leaves researchers with many choices when it comes to pursuing a 'DIY' (do-it-yourself) imaging project. Yet it also means that picking the right tools for a given lab or project can be a daunting task. But the researchers active in this space are enthusiastic about the future this portends for biomedical imaging research. "I think there's a growing community of people who just think that if you're doing science properly, it needs to be transparent and reproducible—and that means you need to share your methods," says Richard Bowman, of the University of Bath in the UK. "And the more of those products that are able to join up, the more powerful this ecosystem becomes."



Vasculature in a cleared and stained mouse brain, imaged with a MesoSPIM instrument. Credit: Fabian Voigt (Harvard University); Nicolas Renier & Thomas Topilko (ICM Paris)

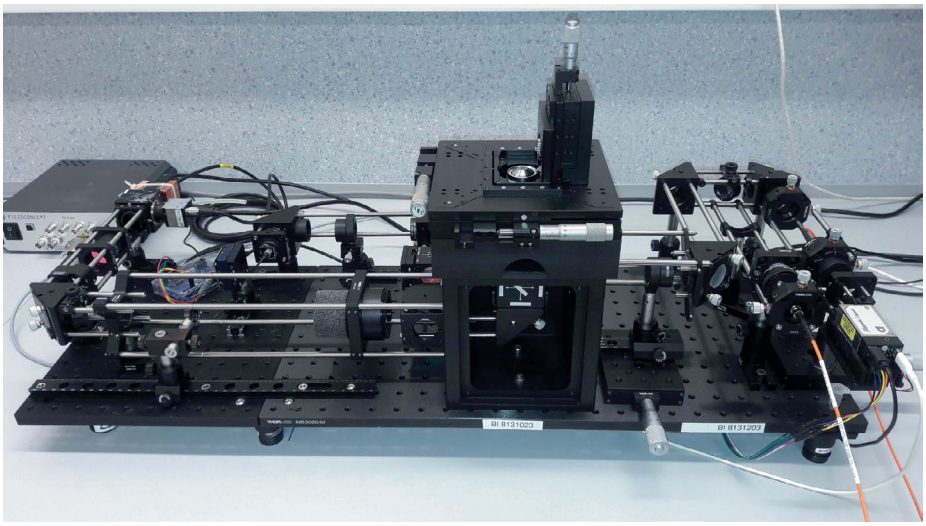
Algorithms for all

The origins of open source in microscopy date back to 1987, when Wayne Rasband, a programmer at the US National Institutes of Health (NIH), began distributing a Macintosh-based tool that he had authored called NIH Image. It was designed for relatively simple tasks, such as demarcating bands on electrophoresis gels, but it quickly accumulated a loyal user base.

"People started downloading it and modifying it for microscopy and other kinds of things," says Eliceiri. Some of the hunger for this software was driven by the lack of robust commercial alternatives, but many also embraced the fact that Rasband made it easy to extend and modify. In the 1990s, NIH Image evolved into ImageJ—where the 'J' refers to the then-newly-created Java programming language—and its vibrant user base continued to grow steadily. Nearly 25 years later, ImageJ has become the tool of choice for labs around the world. "It's inspirational—I would almost say that I went into the bio-image analysis field because of ImageJ," says Ricardo Henriques, of the Instituto Gulbenkian de Ciência in Oeiras, Portugal. "As a developer, I had all the tools to open images, to show those images, to correct the graphical interface, and to create and prototype code very quickly through scripts." Among other tools, Henriques and colleagues have developed a set of plugins called NanoJ, which is specifically designed to facilitate ImageJ analysis of super-resolution microscopy images[1].

Eliceiri credits this level of user engagement with the software's success. ImageJ now boasts upward of 500 plugins, authored by both in-house and community developers, that users can employ to optimize acquisition and analysis for almost any imaginable imaging experiment. The basic ImageJ interface remains fairly spartan, however, so that configuring the software for a given experiment requires some expertise and familiarity with the plugin ecosystem. To simplify this process, Eliceiri teamed up with Pavel Tomancak of the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden and Albert Cardona of the Swiss Federal Institute of Technology Zurich to develop Fiji[2]. This is a distribution of ImageJ that bundles together a hand-picked collection of plugins specifically designed for biological imaging, making it easy for biologists to use the software.

Several other open-source software packages have also become laboratory mainstays. CellProfiler was first developed by Anne Carpenter and colleagues at the Whitehead Institute for Biomedical Research in 2005 as a tool for high-throughput analysis of cell-based assays[3]. Broad Institute researcher Beth Cimini, who currently co-manages CellProfiler, first fell in love with the tool as a wet-lab scientist with little coding expertise. "I didn't really feel comfortable writing a ton of ImageJ macros, but with CellProfiler, I could create a



A 3D-printed MiCube system, assembled by Marijonas Tutkus, Nour Alsamsam and Aurimas Kopūstas at Vilnius University, Lithuania. Credit: Aurimas Kopūstas (Vilnius University)

pipeline file that didn't require any coding, and then give it to my labmates who didn't know any coding at all," she says.

Another popular package is the µManager (Micro-Manager) software, originally developed in 2005 by Nico Stuurman and Nenad Amodaj in Ron Vale's lab at the University of California at San Francisco, which is designed to control the operation of the instrument itself. Using this tool, researchers can issue commands to light sources, filters, cameras, stages and other microscope components to enable the reproducible execution of complex imaging experiments. "We'd love to see a future where the default is that everybody's microscope can run everybody else's protocols," says Bowman. "We're not there yet, but µManager is probably the best attempt at making this happen."

Although these three tools have become ubiquitous, there is also a steady stream of new open-source algorithms bubbling up into preprints and repositories like GitHub. Many of these are more specialized, focusing on cutting-edge techniques for which commercial tools are either not available or not delivering the specific capabilities that researchers need. For example, deep learning and machine learning can make it easier to extract rich insights from vast amounts of image data, but these techniques can be daunting to researchers without coding experience. Henriques' team developed a tool called ZeroCostDL4Mic as a 'gateway drug' for these users, allowing them to leverage existing cloud-based resources to perform tasks like cell segmentation and providing useful analytics to provide feedback on the quality of their deep learning models[4].

Spoiled for choice

This wealth of tools means that imaging labs can readily run their entire operations on open-source software. "We have not been using any commercial imaging software in my lab for years," says Stuurman. But this is not to say that the open-source community is in opposition to the commercial world. Eliceiri notes that the ImageJ team has worked with imaging companies such as Bruker and Zeiss, and Stuurman says that the µManager platform has benefited from close collaboration with major manufacturers of cameras and other mechanized microscope components.

And even the open-source developers themselves see an important role for commercial software in the ecosystem. This is partly a matter of business models, whereby the fees paid by users guarantee ready access to real-time customer support that open-source can't match. And although open-source developers generally offer online resources for users, this toolbox tends to be more dependent on the availability of in-house expertise. "If you want to run completely on open-source software, you need one or two people who are working full time on supporting that and setting up environments where you can do that," says Stuurman.

Cimini notes that some open-source tools also presume a level of comfort with coding that typical users simply don't have. "Most biologists really don't want to go anywhere near a command line," she says, adding that her own early encounters with the relatively user-friendly CellProfiler as a graduate student were somewhat intimidating. Accordingly, the development team for this software always includes a wet-lab biologist as well as a computational expert to ensure that it remains accessible.

Another major issue is interoperability, and getting multiple software tools to integrate into a single pipeline for image acquisition, processing and analysis. The choice of programming language can be a hurdle here. For example, whereas most older software packages were developed for Java, many computational biologists have now transitioned to Python. Multiple efforts are now underway to develop adaptations that allow labs to make use of whichever tools they like, regardless of their coding language preferences, and the Chan-Zuckerberg Initiative (CZI) has provided an important source of financial support here. "CZI has given us a charge to make sure our tools work together," says Eliceiri. "We've been working quite hard on something called PyImageJ, which is to try to make Python fully and bidirectionally accessible from ImageJ." The CZI is also supporting development of a toolbox called napari, which essentially offers a Python-based equivalent to Fiji. But Eliceiri also emphasizes the importance of building bridges between toolboxes rather than trying to port everything over into a single framework. "We need to live in a polyglot world," he says.

Perhaps most daunting of all is the challenge of figuring out which software or plugin to use in the first place. "I think there's easily over 30 or 40 software packages just to do super-resolution reconstructions," says Henriques. He notes that some conferences and communities run challenges that allow head-to-head comparison of the performance of different software packages with various experiment-specific datasets. But a recently launched online community called the Scientific Community Image Forum, or image.sc, offers a more general solution. Image.sc began as a merger between the online communities for ImageJ and CellProfiler, but subsequently expanded to include the Network of European Bioimage Analysts (NEUBIAS) and dozens of other development teams. "Instead of figuring out what tool is right for the job, you can post for all the developers, and they're all there to help you," says Eliceiri.

Bespoke bioimaging

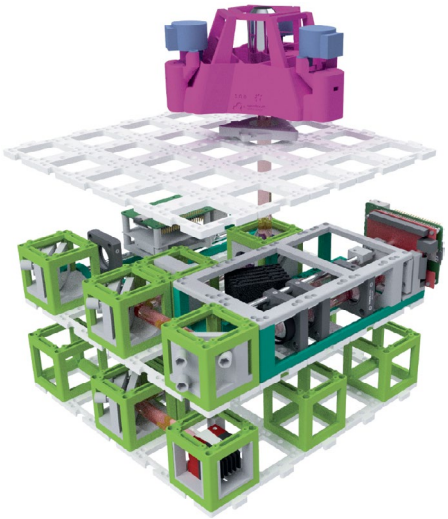
More recently, however, the same core concepts animating the open-source software movement—transparency about methods, community-oriented development and accessibility—have begun percolating into the hardware world. Many labs build their own microscopes, but these are typically one-off designs developed with

in-house expertise, and not intended for broad replication within the scientific community. These open-hardware initiatives collectively target the needs of a wide range of current and aspiring microscopists, although only a relatively small number of such platforms have been developed to date.

In developing the Foldscope device, Prakash's sought to make imaging accessible to literally anybody, from schoolchildren to researchers in the field. "The goal was to literally print microscopes like newspapers," he says. "We developed a process where we can print microscopes in flat construction that are ultra-light and almost indestructible." These devices offer sufficient resolution to image individual bacteria, and Prakash reports that more than 1.5 million have been shipped to users around the world, who have built, modified and adapted the devices for use in hundreds of studies.

Far more sophisticated instruments have also become possible thanks to two major shifts in the hardware ecosystem. One is the ready availability of high-quality electronics and components at ever-falling prices. Vendors such as ThorLabs supply research-grade parts that are widely used for DIY microscopy, and even consumer-grade parts can sometimes do the trick. "Nowadays, I make use of €500 [~$580] cameras that have similar performance to cameras where I paid €10,000 [~$11,500] five or ten years ago," says Johannes Hohlbein, of Wageningen University in the Netherlands. "And a Blu-Ray player for €30 [~$35] has a powerful blue laser built in and the mechanism to move the laser head close enough to a disc with high resolution." Combine this with low-cost computing hardware such as the Raspberry Pi, and one can potentially round up most of the components needed to build an instrument from online marketplaces like eBay or AliExpress. Even precision optical components such as objectives can be obtained at reasonable cost, if the user is prepared to accept trade-offs in image quality.

The second shift is the growing availability of 3D printing. When Henriques began exploring the use of 3D-printed instrument components in his lab, he was startled by how affordable it was. "Even some of the low-end 3D printers, which cost €300 or €400 [~$350–460], often produce plastic parts of sufficient quality for many things that you might want in a lab," he says. This means that parts lists can freely be shared online as digital blueprints, and then fed directly into a 3D printer anywhere in the world. But users also need to be mindful of the limitations of the currently available plastic 'inks', some of which may be especially prone to bending or warping when subjected to heavy loads or temperature fluctuations.



Different open-hardware toolkits can be combined—for example, the OpenFlexure stage can be integrated into the UC2 modular optics toolbox for cost-effective super-resolution imaging. Credit: Benedict Diederich (Leibniz Institute of Photonic Technology)

Multiple groups have now developed modular microscopy systems in which 3D-printed components are combined with off-the-shelf hardware. For example, Hohlbein's group developed the MiCube, in which printed parts are used to mount and configure commercial lenses, lasers and so forth[5]. "The inspiration I had was that it would be nice to have a block where you can mount a couple of additional components in predefined positions to facilitate super-resolution or single-molecule microscopy," says Hohlbein. A basic instrument can be assembled for as little as €20,000 (~$23,000). Another such modular instrument is the UC2 system developed by Benedict Diederich, a postdoc in Rainer Heintzmann's group at the Leibniz Institute of Photonic Technology in Jena, Germany[6]. This comprises building blocks based on 3D-printed cubes and inserts that can be custom configured Lego-style to conduct experiments ranging from conventional brightfield imaging to super-resolution structured illumination microscopy.

Other instruments require minimal assembly by users. Emma Sierecki and Yann Gambin at the University of New South Wales in Australia were looking for an affordable way to bolster their lab's capacity for single-molecule detection, and designed a streamlined instrument called AttoBright[7]. This instrument body can be printed as a monolithic framework incorporating just a single light source and filter, and is intended to offer a simple, low-cost solution for labs that routinely perform single-molecule experiments, such as fluorescence correlation spectroscopy, with a specific optical configuration. "The idea was to remove as much as we could so that there was way less time to align the whole setup, and so anybody basically can do that within five minutes," says Sierecki.

The OpenFlexure instrument developed by Bowman and colleagues is also near-exclusively composed of 3D-printed parts, with the exception of the optics, and is designed to deliver affordable access to automated microscopy to resource-limited laboratories and high-powered institutions alike[8]. "Pretty much the whole microscope prints as a single piece, including all the moving parts," says Bowman. "If you wanted to make this with injection molding, you'd need to make a ton of different parts and then click them all together." OpenFlexure relies on the controlled bending of its plastic parts to achieve movement of components such as the objective, eliminating the need for precision machining, and the whole system can be run from a web interface connected to a Raspberry Pi within the instrument.

The Prakash group's Squid imaging platform relies primarily on machined rather than 3D-printed parts, but his lab has made the CAD blueprints for these components—along with all other resources necessary for assembly—freely available online[9]. Although more expensive than printed parts, these components also confer greater robustness and mechanical precision while still enabling labs to assemble research-grade instruments for as little as $1,000. "When you look at data from Squid, you can really compete with many platforms," says Prakash. "We've demonstrated five to ten different microscopy methods with it." Most recently, his group has been testing Squid's performance with cutting-edge techniques such as spatial transcriptomics, in which RNA expression patterns are visually mapped within specimens.

Imaging for any occasion

Open hardware can offer the best entry point to microscopy for users on a budget, including students having their first encounter with biological imaging. For example, Prakash has put considerable effort into getting Foldscope into classrooms over the years, and Diederich is also working to get UC2 into German schools.

These systems can also be massively enabling for scientists and clinicians in low- and middle-income countries. This

Joram Mduda at the Ifakara Health Institute in Tanzania, with multiple OpenFlexure instruments. Credit: Richard Bowman (University of Bath)

has been a primary goal of the OpenFlexure initiative, and Bowman is working closely with engineers based in Tanzania and elsewhere in Africa to facilitate the distribution of his team's instruments to local laboratories working on malaria diagnosis, as well as other applications. The goal is to build local capacity for manufacturing rather than relying on charity. "You can probably get someone to donate a microscope, but the problem is getting it serviced when it breaks or getting replacement parts or getting the right proprietary consumables," says Bowman. "A ridiculous quantity of equipment is laying idle because it can't be maintained."

The broader research community can also benefit considerably from such open-hardware systems. Sierecki's team routinely performs single-molecule analysis, but her lab only had room for two commercial instruments—leaving some of her researchers idle while those were in use. "At one point, the small room for microscopy that we had was just full," she says. AttoBright enabled her to build out capacity with compact, affordable instruments that also offer a useful vehicle for training new lab members. A lower-cost microscope can also allow routine imaging in conditions where a researcher might shy away from deploying a million-dollar instrument. For example, Prakash has developed Squid instruments for imaging live tuberculosis pathogens within incubators under high-biosafety-level conditions. "In a period of two weeks, we were able to reconfigure the sets of instruments to deploy them in a BSL3 facility," he says.

Open hardware can also provide easier access to imaging techniques that are out of reach because of technical barriers. For example, Sierecki sees AttoBright as an enabling tool for labs that want to venture into single-molecule analysis for the first time.

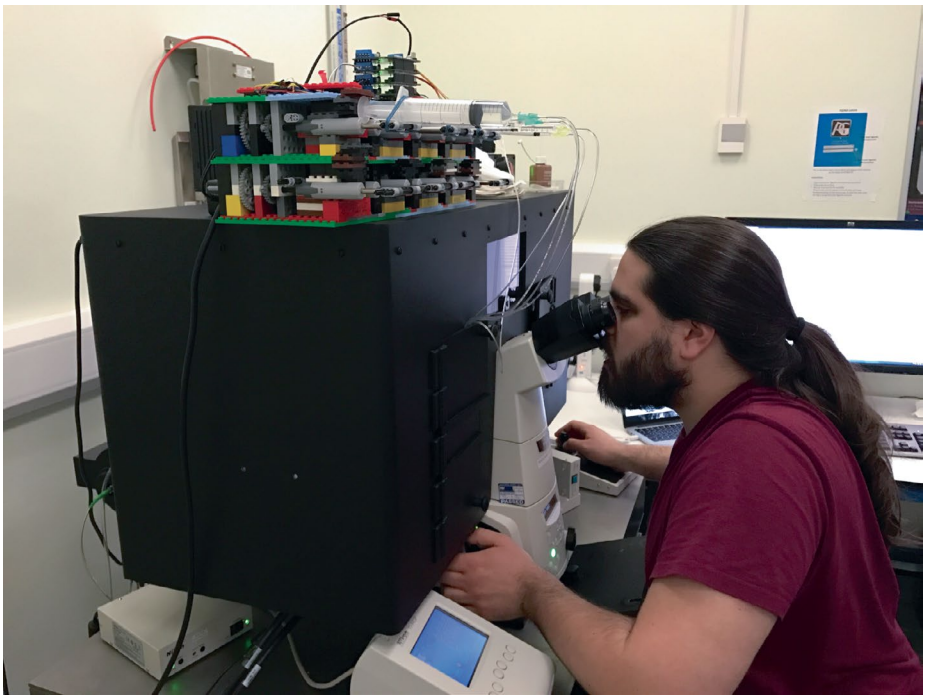
Multiple initiatives have also sprung up to help labs get started with light-sheet microscopy—a powerful tool for imaging large samples in three dimensions, but one requiring highly specialized instrumentation. The OpenSPIM project was launched by Tomancak and colleagues in 2013 to guide and support research labs in the design and construction of 'entry-level' light-sheet instruments. More recently, Fabian Voigt and colleagues in Fritjof Helmchen's group at the University of Zurich set up a parallel initiative called MesoSPIM, which provides blueprints and other documentation for cutting-edge instruments that can rapidly capture high-quality data from chemically cleared tissue samples[10]. "We can image a mouse brain with five- or six-micron resolution within six or seven minutes," says Voigt, who is now a postdoc at Harvard University. MesoSPIM isn't cheap—a high-end design can cost up to €250,000 (~$290,000), compared to €30,000–50,000 (~$35,000–$60,000) for an OpenSPIM—but Voigt notes that it can deliver capabilities that match those of commercial instruments costing up to three times as much.

It takes a community

Open-software and -hardware initiatives must both struggle with similar challenges to succeed. One is the need for meticulous documentation to get new users started and to help them through the inevitable snags that occur when onboarding any new tool. "The majority of people just don't have time, so you really need to break it down to very tiny details," says Diederich. Close contact with users online is also essential, as this can enable the identification of unmet needs that could become future features, or reveal underappreciated snags in a program's or instrument's design. "There's nothing like just watching somebody who's using the software for the first time to tell you what are the pain points and what are the things that could be clearer," says Cimini. Dedicated internet forums and GitHub pages or wikis also create communities where users can share their own hacks and customizations.

Without long-term support and maintenance, even a truly inspired tool will fall by the wayside, and this remains a major challenge for both software and hardware developers. Voigt notes that when he left the University of Zurich, the Helmchen lab was able to obtain funding to hire dedicated support staff for MesoSPIM over the next several years—but such support is sadly rare. "Development, maintenance and outreach often get overshadowed, and that's just because there are not many grant designs that allow that," says Eliceiri. Stuurman concurs, noting that without the CZI, there would be few alternatives for keeping µManager funded. "Public money seems to have completely dried up," he says. As such, the survival and success of open-microscopy efforts are directly dependent on the passion, resources and free time of their creators.

But for now, the community remains energized and focused on ways to band together to make use of their collective resources. Open-hardware and open-software efforts already routinely intersect, with many DIY microscopes making use of tools like Fiji and µManager, but even more extensive cross-pollination is also in the works. "We are trying to merge the best of all the projects," says Diederich. "For example, having the very nice stages from Richard Bowman's group while adding the ability for super-resolution imaging with our cubes."



Pedro Almada uses an open-source syringe-pump array built out of Lego, 3D-printed components and Arduino-based electronics for fluorescence microscopy. Credit: Ricardo Henriques (Laboratory for Molecular Cell Biology, UCL)

And in the long run, the impact of microscopy-on-demand could be truly transformative—in terms of people who can engage with imaging for the first time, but also in terms of work that can be taken out of the hands of people altogether. "Oftentimes huge amounts of smart people's time are wasted doing very boring, repetitive things," says Bowman. For example, many live cell studies require extensive imaging to observe rare biological events, or acquisition of vast datasets to train machine-learning algorithms to recognize features of interest. 'Farms' comprising dozens of low-cost automated microscopes working in tandem could offer an affordable solution that lets scientists stay focused on experimental design and analysis rather than getting bogged down in execution. "The easiest way to do that is to just have a whole rack of microscopes running 24/7," says Bowman. "We're not there yet, but I think that's very much the sort of thing that we would like to see." ❐

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