Comment

Open microscopy in the life sciences: Quo Vadis?

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Abstract

Light microscopy is playing a fundamental role in the life sciences enabling researchers to observe cellular mechanisms with high spatial and temporal resolution. The increasing complexity of current imaging technologies coupled with financial constraints of potential users, however, hamper the general accessibility and potential reach of cutting-edge microscopy. Open microscopy can address this issue by making well-designed and well-documented hardware and software solutions openly available to a broad audience. In this comment, we provide a definition of open microscopy and present recent projects in the field. We discuss current and future challenges of open microscopy and their implications for funders, policymakers, researchers, and scientists. We believe that open microscopy requires a holistic approach. Sample preparation, designing and building of hardware components, writing software, data acquisition, and data interpretation must go hand in hand to enable interdisciplinary and reproducible science to the benefit of society.

A. Introduction

Open science seeks to improve transparency, reproducibility, inclusiveness, and accessibility of research and innovation[1]. This is important because, in our opinion, academia still has a tendency to keep the science behind closed doors (Figure 1). Until recently, most results were published in journals inaccessible to most citizens. Access to information on specific methodologies, experimental settings or raw data was, and often still is, largely dependent upon the courtesy of the author's post-publication. Open science is challenging these restrictions by providing additional interaction points between researchers and citizens. For scientific data, the FAIR principle (Findable, Accessible, Interoperable, Reusable)[2] provides guidelines for moving science towards being “shared knowledge accessible to all”. With that in mind, and following a previous definition of open science[3], we define open microscopy as a movement to make scientific research involving microscopy, any associated data and dissemination thereof accessible to all levels of an inquiring society. Specifically, we define associated data as information on (i) how to build, use and maintain microscopes (hardware), (ii) how to prepare, handle and measure samples (assays) and (iii) how to analyze, distribute and store experimental data and computational models (software). Note that we here define assays broadly referring to samples and everything in addition to hardware and software that enables meaningful experiments.

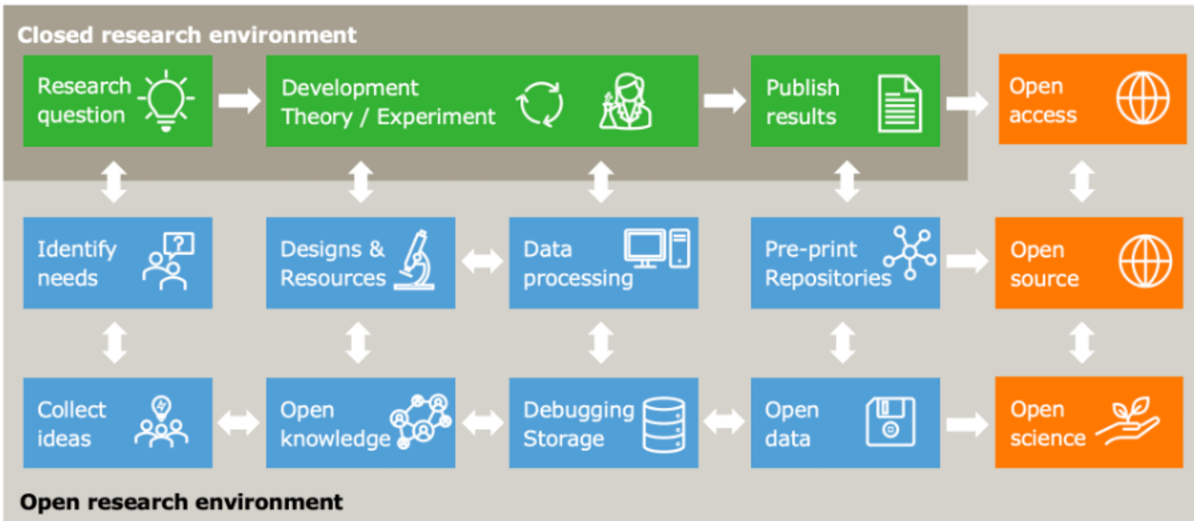

Figure 1. Closed research environments are defined by strong gatekeeping within individual labs, research units or scientific areas: research questions are chosen by individuals, research is undertaken by specialists and results are published in journals inaccessible to the general public. In an idealized open research environment, the unrestricted flow of information and exchange of ideas, resources and data is both facilitated and encouraged. Consequently, this efficient pooling of resources supports further scientific progress.

Light microscopy has been pivotal in the life sciences to study small features and objects otherwise hidden to the naked eye. Simple microscopes such as the 2$ Foldscope or smartphone auxiliary lenses are forming the basis of citizen science projects, scientific education, and medical diagnosis[4,5]. Driven by a societal and academic shift towards open science and technology, more and more information on advanced microscopy has become publicly available[6]. While open software can be downloaded directly, the development of open hardware accelerated only recently with the increasing accessibility and affordability of suitable hardware components. With low-cost 3D printers, rapid reproduction of designs and prototyping moved from professional machine shops to the hobby room. Designs milled from solid aluminum are ordered via web shops and delivered within days. Mass-produced electronics such as light-emitting or laser diodes, microcontrollers, lenses, and industrial cameras have further reduced the costs and time requirements of building complex instrumentation. Scientific-grade components such as laser engines, objectives, and low noise cameras have been successfully replaced by cheaper alternatives[7–10].

All open projects empower scientists and researchers to adopt solutions - even if only as a source of inspiration. In our experience, open hardware and software projects help to keep research going at a time of fierce competition for limited funding. Projects that have developed strong communities provide support within minutes in public forums and over social media. In the following, we will briefly highlight current open-microscopy projects, and discuss opportunities and challenges that we consider important to ensure the continuing growth and future success of open microscopy.

B. On the purpose of open microscopy

B.1. Open microscopy as an example for good scientific practice

Even for specialist labs, implementing hardware-based imaging modalities that are published without sufficient documentation requires extensive reverse engineering and tinkering instead of waiting for commercial suppliers to implement new modalities. While commercial microscopes feature safety measures, warranty, and further support, many contain proprietary information with specific internal settings and characteristics remaining unknown to the user. Open microscopy can overcome this problem by ensuring that any new method, both hardware and software, is sufficiently documented and open to allow straightforward implementation and replication. In this process, the sharing of materials or information between two or more parties should not be hindered by restrictive material transfer/non-disclosure agreements. For open hardware, recent work highlights general opportunities and best practices[11,12]. For light microscopy, this can include documenting the assembly and manufacturing and providing guides, bill of materials (BOM) and video tutorials.

We argue here that any scientific publication of a new microscopy modality should meet modern standards of scientific reproducibility further discussed in section C2. Detailed documentation, including for example why a feature was implemented in the suggested way, enables others to learn about the given technique and to later explore potential optimization steps. For small hardware or software components, this implies making conceptual drawings or source code available noting that this documentation can even be written in the form of citable scientific publications[13].

We strongly encourage publishers to support scientists who are willing to openly share their designs and work. Support is given by providing guidelines and templates as exemplified by HardwareX (https://zenodo.org/record/3364475) and the Journal of Open-Source Hardware (JOSH). Publishers and editors should further request authors to make their data and code publicly available. We note that the full reproducibility of research is important as without rigorous verification of results and discoveries scientific progress is threatened[14,15].

Academic researchers should be aware that, by default, everything developed and created is the property of the research institute, meaning that researchers leaving the institute may lose both rights and access to their unpublished intellectual contributions. To permit the use and further development of open microscopy projects by anyone, regardless of location or affiliation, we advise choosing appropriate licenses such as CERN Open Hardware License[16], MIT[17], GPL v3[18] or Creative Commons[19]. This also addresses the issue posed by active patents that theoretically can prohibit the use of methodologies in the laboratory[20]. We recommend scientists and developers to make themselves aware of the regulations and possibilities with the institutional IP handling offices.

While not an intrinsic feature of open source, we encourage developers to use version control tools like Git (GitHub, Gitlab) at any stage of the project to share ideas and experimental designs, document the process, and track individual contributions.

B.2. Open microscopy enables flexible and powerful platforms for life scientists

Until recently, microscopy *hardware* developers seeking to develop optical methods faced the choice of either retrofitting new hardware onto an existing commercial microscope or designing and building an entire bespoke microscope from individual components. Monolithic, commercial bodies offer a stable mechanical base and are designed to minimize optical aberrations. Critical optical planes or individual optical components (mirrors, lenses), however, are not easily accessible. Features implemented for user-friendliness and safety (eyepieces, safety interlocks, dedicated software) further limit developers from modifying a setup. Fully customized microscopy designs, on the other hand, offer wider control and more accessibility, but come with their own caveats. Developing new hardware can take a lot of time, especially when used on re-implementing basic components and features such as focusing or sample positioning. Moreover, custom microscopy solutions are often less user-friendly.

In terms of open-microscopy hardware frameworks minimalistic microscopes such as FlyPi[21], OpenFlexure[22], UC2 system[23], µCube[24], Octopi[25] have started changing advanced microscopy from a scarce resource to everyday tools of life scientists and hobby enthusiasts alike (Figure 2). These microscopes are specifically designed to be affordable, adaptable, reproducible, and easily repairable, for example using 3D printed parts instead of specialist components as recently reviewed[26].

For researchers interested in volumetric imaging, the OpenSPIM (SPIM: Selective Plane Illumination Microscopy) project enabled many labs to build, apply and teach light sheet microscopy at a time when commercial solutions were neither accessible nor affordable[27]. Similarly, the mesoSPIM initiative provides comprehensive open-source documentation[28] and detailed protocols for tissue clearing[29]. Further, Scanned Oblique Plane illumination microscopy (SOPi) was introduced that features open hardware assembly, an alignment protocol, and control software for single-objective light sheet microscopy[30].

Other microscopy frameworks resemble more closely the layout of conventional upright commercial systems but feature a higher degree of modularity and customizability (Figure 2). The frameworks enable single-molecule localization microscopy (WOSM[31], liteTIRF[32], miCube[33]), epifluorescence microscopy (LFSM[34]), high-throughput screening and tracking of microorganisms (Squid[8] or [35]), diffusion-based confocal microscopy (smFRETbox)[36] or detection of protein aggregation[37], two-photon Ca^{2+} deep tissue imaging[38], and structured illumination microscopy for sub-diffraction resolved (live-) cell imaging[39,40].

Depending on exact implementations, the cost of these frameworks can be considerably lower than for commercial systems (UC2[23] and Squid[8] < 2k Euros, miCube[33] <100k Euros) although, as discussed below, the costs due to expert time investment for both building and maintenance should be taken into account.

Python-based software solutions for image processing[41–44] and image acquisition are enriching the long-dominant JAVA-based programs ImageJ[45]/Fiji[46] and µManager[47] prospectively. The manufacturer- and platform-independent file format of the Open Microscopy Environment (OME) initiative[48] ensures long-term data compatibility, e.g., in the growing field of deep learning for image quality improvements, segmentation and overall data analysis (e.g., CARE[49], StarDist[50], CellPose[51], QuPath[52], ZeroCostDL4Mic[53]) as recently discussed[13,54,55].

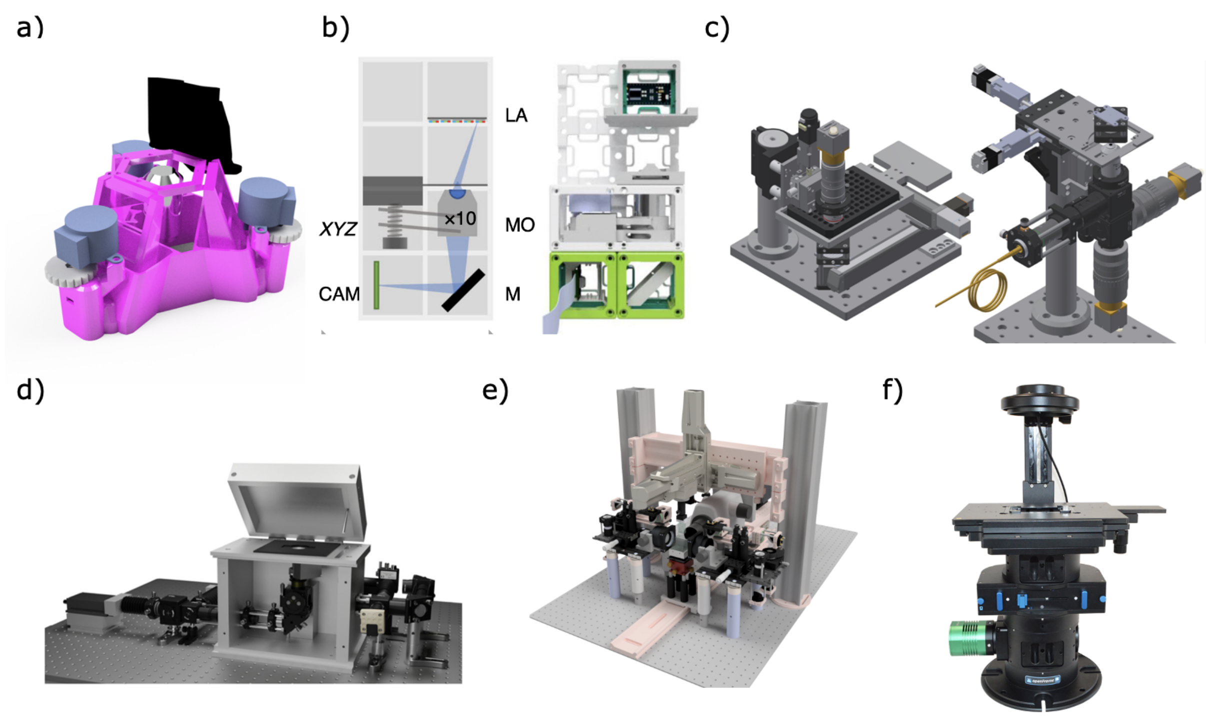

Figure 2. Overview of open-microscopy hardware projects. **a)** OpenFlexure devices enable 3D printed microscopes with high mechanical stability controllable via a web browser[22]. **b)** “You see, too” (UC2) is a general-purpose modular framework for interactive (electro)-optical projects[23]. **c)** The Squid platform represents a full suite of hardware and software components for rapidly configuring high-performance microscopes[8]. **d)** The single-molecule Förster Resonance Energy Transfer box

(smFRETbox) enables diffusion-based measurements of individual biomolecules[36]. **e)** The MesoSPIM project presents open-hardware microscopy platforms for imaging cleared tissue[28]. **f)** The openFrame is a commercially available open microscopy framework[56]. Permissions for images: a) adapted (new rendering under CC-BY 4.0); b) and d) reproduced under CC-BY 4.0, c) reproduced under CC-BY-NC-ND 4.0, e) adapted (new rendering under CC-BY-NC-ND 4.0) and f) reproduced with permission from Cairn-Research).

C. Current and future challenges

Open science and open microscopy create plenty of opportunities for researchers and users by facilitating new innovations, increasing the accessibility of microscopy, and enabling better reproducibility of scientific research. In this section, we will look into the future of open microscopy and critically discuss current limitations.

C.1. Accessibility, availability, safety, and time versus money

The reasons for working on open-microscopy projects are as diverse as the people involved. Some might enjoy the tinkering aspects most (*the developer*), others use open tools to address their scientific questions as affordable and fitting as possible (*the end-user*). Developers and end-users, and all the researchers falling somewhere in between, may have different visions for open microscopy and should be aware of each other. The end-user is likely to prefer more polished software or hardware, sometimes even willing to sacrifice additional features for stability and ease of use. Some end-users might have less time to build or adapt complete solutions and would rather prefer to buy them. Both sides ultimately depend on each other like in a classical “supply and demand” situation in which a growing request for innovative solutions can support people working on them.

One frequently encountered statement is that an open microscope was built for costs that are cheaper than the price of a comparable commercially available instrument. We consider such statements at best misleading as neither the costs of development nor the time spent to build the instrument is properly accounted for. We also point out that any company must fulfill a minimum of conformity with health, safety, and environmental protection standards (e.g., CE, FCC, TÜV or others) for their products and provide customer support. In open projects, even when using commercially available components, the sole responsibility for safety is shifted to the user. Additionally, user support depends on the goodwill and the spare time of the developers. We urge users of open microscopy to pay attention to safety in the widest sense, especially when dealing with optical components such as high-power laser diodes that can cause physical harm. We recommend working closely together with local safety officers.

C.2. Standards and continuing proliferation

With the number of hardware and software frameworks rapidly increasing, new challenges arise as potential users might feel overwhelmed by the number of available options. An illustrative example of proliferation is the variety of software packages available for data analysis in single-molecule localization microscopy. Here, the curated evaluation of over 30 different software packages using a diverse set of metrics highlighted the benefits of open microscopy[57]: Open packages can be directly compared to each other by everyone, helping end-users to freely choose data analysis software optimal for their environment in terms of accuracy, speed robustness, reliability, and user-friendliness. We conclude that proliferation should be seen as an opportunity rather than a threat pointing out a recent series of documents on the implementation of standards in open hardware and software development[58] as well as data provenance and quality control in microscopy[59–62]. We suggest that these best practices are requested and followed by scientists, reviewers, and editors to enable long-lasting inter-device operability.

C.3. The challenge of generating shareable hardware files

Whereas many file formats for storing and analyzing images are open and suitable viewers are freely available, this is not necessarily the case for hardware designs that feature Computer Aided Design (CAD)[63]. For 3D printing, 3D models exported in the *.stl format describes only the surface geometry of a three-dimensional object without any scale thereby inhibiting any modifications to the design. Alternatives, such as sharing links to cloud-based CAD software (e.g., Fusion360, Tinkercad) or relying on open-source CAD models (e.g., openSCAD or FreeCAD) can help to distribute design files across different development environments. Ultimately, publishers and developers should ensure that design files are available in formats as proposed by the open source hardware association (OSHWA) (https://www.oshwa.org/sharing-best-practices/).

C.4. Connecting open-source software to open hardware

The close connection between open hardware and software is inevitable for complex microscopy projects. Projects such as µManager[47], Pycro-Manager[41] and Python-microscopy[42,64] have been playing a key role in connecting setup control, data acquisition and data analysis. When it comes to hardware control, the availability of open-source device drivers and adapters is crucial. The software architecture used in µManager[47], for example, standardizes how hardware devices can be controlled from diverse software components via a plug-in mechanism, making it easier for developers to contribute plugins. As a case in point, the µManager community managed to collect hundreds of device adapters (https://micro-manager.org/Device_Support).

Combining open software solutions for microscope control, image processing and data analysis is hugely challenging, requiring developers from different backgrounds closely working together to optimize signal and data streams. Promising steps towards “smart microscopy” have been made, namely by the software autopilot[65] and by combining OpenFlexure, ImJoy and UC2[66]. Overall, developing algorithms for plugin-based software projects allow easy sharing with the community; algorithms and code can thus be used without much prior knowledge leading to faster acceptance by users.

C.5. Strategies to enable long term support of open-microscopy projects

From our experience, open-microscopy projects are often initially driven by one or two people. Most projects have a limited lifetime as scientific advancements and new hardware or software can quickly render entire projects obsolete. Other projects develop into large community-driven projects with enduring relevance and impact. We advise clear communication with the potential target audience to keep expectations aligned and in check: Developers should indicate as soon as possible whether their project is intended as a research platform for others that could turn into a community-driven project or whether the developer is mainly interested in using their hardware or software to promote their own research. Communication channels such as online forums (Discourse, ImageSc), Slack/MS Teams/Discord channels, online seminars and Github/Gitlab issue pages, enable a direct way of interaction between users and developers, a crucial feature of community-driven projects.

For the primary developer, providing this kind of service plus managing the contributions of others comes at substantial costs, which are often difficult to cover in the current academic incentive system and put a strain, especially on smaller labs. Although funding bodies such as NSF, NIH, Wellcome Trust, and Max Planck Society nowadays widely propagate the idea of open science, institutional support or open calls that are explicitly dedicated to the development and continuation of open hardware, software, and knowledge exchange projects are still rare. The Chan Zuckerberg Initiative and NASA are notable exceptions providing substantial funding to support open science. We urge policy makers and funders to set up additional funding schemes supporting new as well as existing open-microscopy projects. Many projects will benefit from small grants (25k Euros), e.g., for designing injection molds for the UC2 system to produce mounting cubes (Fig. 2b). Larger grants could be used to hire programmers to increase both functionality and accessibility of popular software packages. In addition to the direct funding of projects, we further highlight the importance of having 3D printers, CNC machines and general know-how on electronics or mechanical and optical engineering available at universities and other knowledge institutions. Local workshops are perfectly suited for the task of maintaining knowledge and expertise.

Furthermore, interacting with the community, selecting issues to work on and motivating others to support open microscopy requires a substantial investment of time and effort. We recommend developers to think about these aspects carefully and identify supporting resources and people at an early stage; follow-up costs, both in time and money, cannot be paid by a single PhD student or postdoc no matter how enthusiastic they are.

What are the requirements for open microscopy projects to succeed as community standards? Although none of the guidelines mentioned in **Box 1** is strictly essential, successful projects such as OpenFlexure and UC2 fulfill many of them.

BOX 1. Guidelines for open microscopy

Uniqueness. Any new project should bring a new approach to the table, differentiating it sufficiently from existing projects. Defined broadly, uniqueness could include substantially reduced costs, higher mechanical stability, higher optical resolution, faster analysis, or better visualization. If uniqueness is lacking, we recommend contributing to existing projects.

Resources: To ensure continuity of open projects, one or more core developers with sufficient resources in terms of time, money, or appreciation are required.

Involvement: Developers should strive to create and maintain an active user base on all levels of involvement ranging from "use as is", "test and report bugs", "request features" to "fix bugs and implement small features" or even "write new add-ons". "Open source" should never be translated as "free support". Projects build a strong community when their users can get a feeling of empowerment.

Documentation. Detailed documentation is key for new users and developers to join and potentially continue a project even if initial contributors left or initial investments have run dry.

Interoperability. Developers should strive for device interoperability by means of openly developed interfaces.

Need. For each new project, a clear need should be identified by the developer/community. The community-driven development of napari[67], was kickstarted by the wish to have an adaptable multi-dimensional image viewer available in Python. The project is now receiving substantial support from the Chan Zuckerberg Initiative.

Expertise. The merging of expertise by means of adapting hardware or software designs from different projects can speed up development processes.

We note that larger imaging facilities are well suited to support developers and users. We hope that universities and funders recognize the potential value of having a wide portfolio of maintained open microscopy projects.

C.6. Commercialization of open-source projects

We consider it desirable if hardware projects can make parts or assemblies commercially available. We see an increasing demand for affordable and proven solutions by end-users who are not interested in building scientific instrumentation. In the simplest case, 3D printed or CNC-milled entities (e.g., OpenFlexure or miCube) or assemblies are sold directly or in the form of do-it-yourself kits similar to kits available from Thorlabs, Cairn and others. In special cases, entire microscopy solutions could become user-ready products. For this route of commercialization, however, there are several points to consider.

- Investors required to finance the transition from a prototype to a full product generally prefer solutions that can be protected by patents.
- Within universities, huge overhead costs often make the exploration of commercialization expensive and time-consuming.
- The size of the market might be too small to get sufficient return on investment to keep a small business viable in the long run.
- There is the risk that potential patent infringement is targeted aggressively by established companies as soon as patented technology leaves the realm of pure academic use.
- Academics often lack the knowledge in the areas of business development on how to turn a project from a prototype into a commercially viable and safe product.
- Academics are often reluctant or not able to devote part of their time to setting up a business.

There is a need for universities and their technology transfer units to develop solutions that allow open source hardware to reach the market with minimal bureaucratic and financial overhead for involved researchers. One potential route is involving external companies specializing in the commercialization of academic ideas and products (e.g., Idylle, LabMaker).

Another example is the openFrame microscope developed by the French group and commercially available via Cairn research (https://www.cairn-research.co.uk/product/openframe-microscope/). Some business models and companies even permit the production and sale of open-source hardware under open-source hardware licenses such as the CERN Open Hardware License. For a discussion on potential business models, the reader is referred to Josuah Pearce's essay[68].

When thinking about routes towards commercialization, another business opportunity could be to provide services related to specific open microscopy projects. Scientists who prefer to work with open solutions may neither have the experience nor the time to do these modifications and extensions themselves. Inviting a developer as a guest scientist or consultant might be more effective than hiring a postdoc. Such a job profile, however, still needs to be established and supported by research institutions.

In general, the open hardware field strongly requires role models; people that go from open source to commercialization and talk about it. Conferences, as well as journals, should invite people to talk and write about these important topics showing that open-source business models can be sustainable.

C.7. Continuing training and education

The increasing complexity of methods and tools used in the life sciences requires continuing training and education. The financial investment necessary for hands-on training in optics and related fields has been substantially reduced with open instrumentation and simplified hardware. Moreover, in the interdisciplinary area of microscopy, project-based courses encourage creativity and the development of new approaches to solving individual user problems. Open education in microscopy further improves hardware projects via bidirectional exchange of knowledge and experience.

With the widespread use of digital teaching and learning platforms, and the possibility of building the microscope yourself or converting a smartphone into one, training no longer has to take place at one location. Like in the flipped classroom concept, the tasks are discussed first, possibly online, solved individually outside the classroom and the results are discussed afterwards. During the SARS-CoV-2 pandemic, for example, the possibility of distributing UC2 boxes offered a hands-on practical course at times at which in-person lectures and lab work were not possible.

Low-cost microscopes enable discoveries that can be shared and discussed both in class and with the wider community, e.g., on social networks. The associated ease of access to these tools, which are available to virtually everyone and everywhere, makes education more inclusive and supports the growing interest in STEM subjects. Training young interdisciplinary professionals with the help of open-source tools promote and create international cooperation. An important element for the future is making the resources comprehensive to reduce the burden on educators and provide the easiest possible access for direct use in the classroom.

D. Conclusion

In the past, quantitative light microscopy was seen as an expensive endeavor for specialists. Open microscopy, similar to the open-source movement as a whole, is helping to overcome barriers that prevent scientists and researchers from utilizing and contributing to cutting-edge microscopy-driven research as well as applying microscopy in education.

Method developers pushing the technical limits of microscopy benefit from open microscopy. Instead of expending limited research time reproducing poorly documented systems, they can focus on the genuine novelty in their project. Thus, detailed documentation as required from our earlier definition of open microscopy drives the development of new microscope technologies. Every new project will strongly benefit from the availability and accessibility of smart and open solutions for hardware, software, and assays. In fact, we expect any future cutting edge microscope development to rely on open science in one way or another.

For the large pool of microscopists for whom biological discovery is the key driver, the goal is not necessarily to apply the method with the best resolution. Rather, the aim is to find or develop the most suitable technique, or combination of techniques, that work within the constraints of a specific biological question. These researchers benefit from the modular nature of open microscopy where they can rapidly test, prototype, and tailor different microscopy approaches for their specific system, and often combine multiple techniques in a way that simply would not be feasible in either commercial or traditional home-built systems. This allows researchers to use the best microscopy tool for their project, instead of being limited by what is available in their local facility or needing to embark on multi-year fundraising efforts. Above all, open microscopy opens up the black box of technology-driven device development and makes it more accessible to those who use it. Openly sharing ideas and resources should ultimately inspire users and researchers thereby fostering the development of new imaging methodologies. At its best open microscopy empowers scientific curiosity, creativity, and collaboration. For this reason alone, it is worth investing time and money into its bright future.

Additional resources

A list of hardware and software projects, repositories and additional resources can be found on https://github.com/HohlbeinLab/OpenMicroscopy. The authors welcome contributions to make the list comprehensive and keep the list up to date.

Author contributions

J.H. and K.P. initiated the manuscript. All authors provided sections and contributed to the final version of the text.

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Competing interest

S.H. has an ongoing collaboration and supervises a joint MRC CASE PhD studentship relating to open microscopy with Cairn UK Ltd. All other authors declare no competing interests.

Data availability

A list of hardware and software projects, repositories and additional resources can be found on https://github.com/HohlbeinLab/OpenMicroscopy.

Code availability

Not applicable.

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Figures

Figure 1

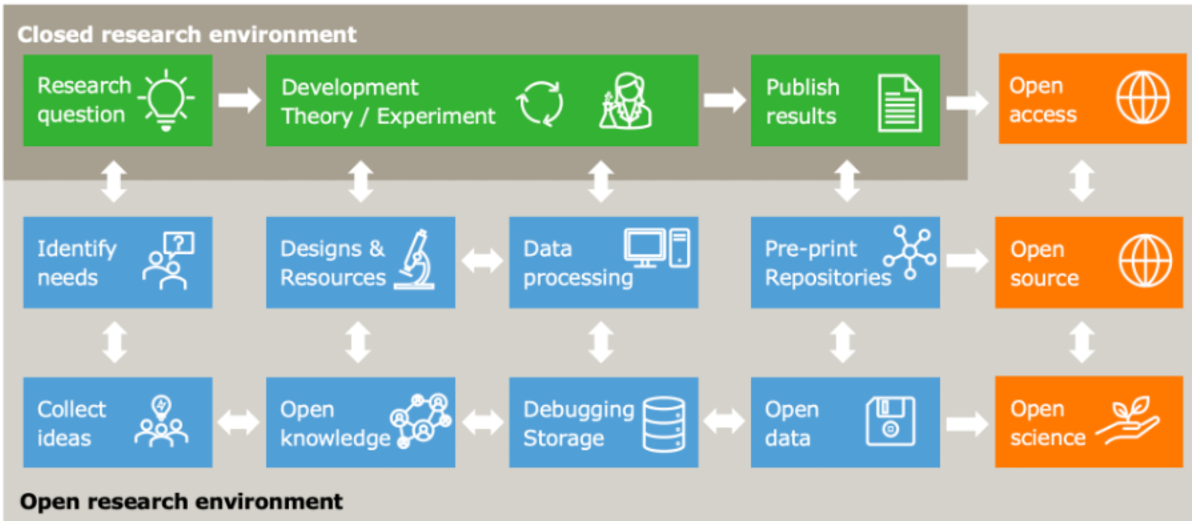

Figure 1. Closed research environments are defined by strong gate-keeping within individual labs, research units or scientific areas: Research questions are chosen by individuals, research is undertaken by specialists and results are published in journals inaccessible to the general public. In an idealized open research environment, the unrestricted flow of information and exchange of ideas, resources and data is both facilitated and encouraged. Consequently, this efficient pooling of resources supports further scientific progress.

Figure 2

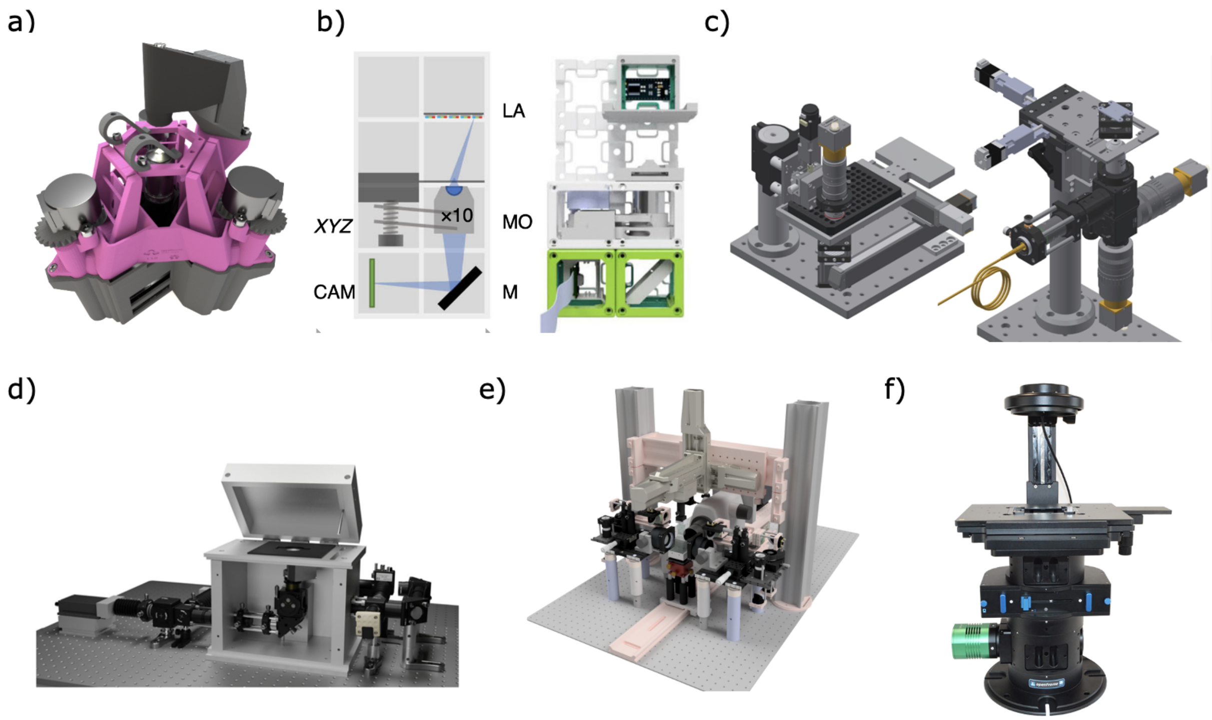

Figure 2. Overview on open-microscopy hardware projects. **a)** OpenFlexure devices enable 3D printed microscopes with high mechanical stability controllable via a web browser[22]. **b)** “You see, too” (UC2) is a general-purpose modular framework for interactive (electro)-optical projects[23]. **c)** The Squid platform represents a full suite of hardware and software components for rapidly configuring high-performance microscopes[8]. **d)** The single-molecule Förster resonance energy transfer box (smFRETbox) enables diffusion-based measurements of individual biomolecules[36]. **e)** The MesoSPIM project presents open-hardware microscopy platforms for imaging cleared tissue[28]. **f)** The openFrame is a commercially available open microscopy framework[56]. Permissions: images a), b), d) reproduced under CC-BY 4.0, c), e) under CC-BY-NC-ND 4.0 and f) with permission from Cairn-Research).