The Field Guide to 3D Printing in Optical Microscopy for Life Sciences

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The maker movement has reached the optics labs, empowering researchers to create and modify microscope designs and imaging accessories. 3D printing has a disruptive impact on the field, improving accessibility to fabrication technologies in additive manufacturing. This approach is particularly useful for rapid, low-cost prototyping, allowing unprecedented levels of productivity and accessibility. From inexpensive microscopes for education such as the FlyPi to the highly complex robotic microscope OpenFlexure, 3D printing is paving the way for the democratization of technology, promoting collaborative environments between researchers, as 3D designs are easily shared. This holds the unique possibility of extending the open-access concept from knowledge to technology, allowing researchers everywhere to use and extend model structures. Here, it is presented a review of additive manufacturing applications in optical microscopy for life sciences, guiding the user through this new and exciting technology and providing a starting point to anyone willing to employ this versatile and powerful new tool.

1. Introduction

From the first optical microscopes invented in the late 16th century to the most recent iterations that can resolve targets beyond the diffraction limit of light, microscopy-based approaches represent a critical tool to study biological phenomena.[1] Recent years have seen a sharp increase in bespoke microscopes, achieving excellent results where commercial solutions were ineffective, for example,

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the Warwick Open Source Microscope (WOSM) (https://wosmic.org) and the OMX Microscope.[2] However, researchers are often limited to commercially available microscopes, as manufacturing microscope components is expensive and slow reliably. This limitation hampers the diversity of new designs, ultimately restricting the innovation of new technology.

The approach of 3D printing holds great potential in this regard as it has had a disruptive impact on manufacturing. This impact is based on its rapid prototyping approach in creating physical objects through additive manufacturing. Additive manufacturing has seen a massive rise in popularity in recent years, from its start as an industrial prototyping tool to current domestic use. It is employed across many settings, from the user printing miniature figurines using common polymers in a small domestic printer[3] to entire houses using concrete in huge industrial machines.[4] In 3D printing, a structure is built bottom-up in an additive manner by depositing sub-millimeter layers of material. In contrast, conventional manufacturing processes usually rely on manual labor and automated processes such as casting, forming, and machining by either subtracting material from a larger starting piece or using molds to shape an object.[5] The additive manufacturing approach pursued in 3D printing uses simplified one-step manufacturing processes, reducing material waste. This is not always attainable in practice, and additional steps to polish a print can be required. Nonetheless, even in cases of failed print jobs, recycling materials is possible.[6,7] On the other hand, subtractive fabrication methods such as milling can produce waste as high as 90%.[8]

In scientific research, additive manufacturing is rapidly becoming a critical tool allowing the rapid development of new designs and the prototyping of machine components with an unprecedented speed for chemical, pharmaceutical, and biological applications.[9–12] Beyond this, 3D printed components can be assembled to create complex machinery such as microscopes,[13] which are the focus of this review. Besides the advantage of rapid manufacturing, 3D printing facilitates novel and powerful approaches to solving problems in science, as it provides ample room for creativity. Additionally, 3D printing communities are well established, promoting collaborations between researchers and even the general public. 3D printing holds the unique possibility for optical microscopy in life science to extend the open-access concept from knowledge to technology, allowing researchers and people with a keen interest in

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science to use, iterate designs, and adapt existing ones for different projects with a high degree of customization.

2. What is 3D Printing?

3D printing is a process that creates a physical object by adding layers of material to recreate a 3D digital object. **Figure 1** shows the typical framework used in 3D printing. This technology involves three main steps: the digital design of a 3D object, the computation of the printing instructions required by the printer, and finally, the fabrication of said object by adding patterned layers of new material. Depending on the application,
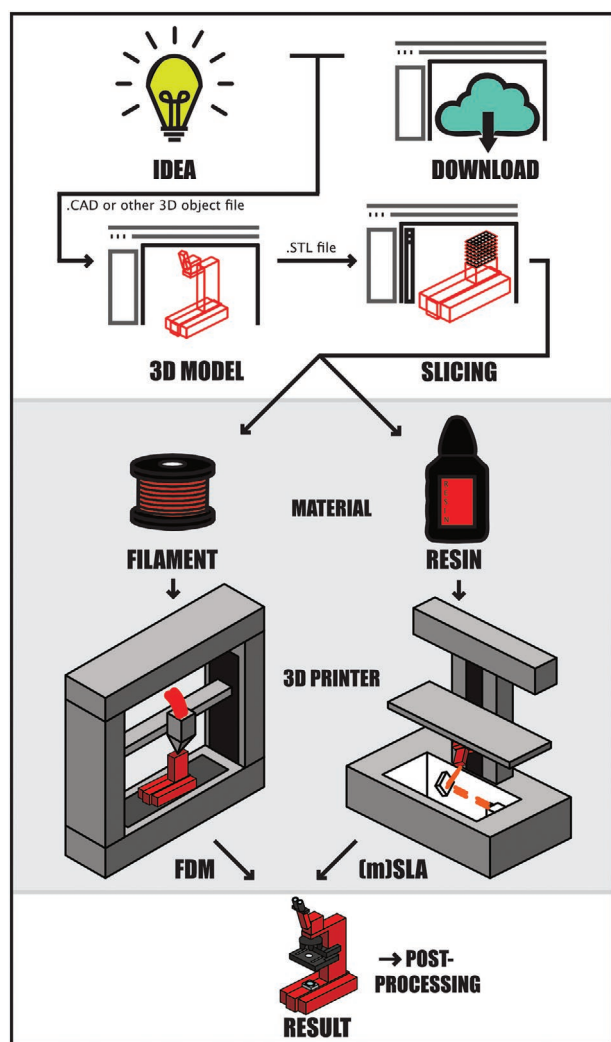
vastly different 3D printing technologies that use different materials can be employed. This review focuses on two of the most popular methods: fused deposition modelling (FDM) and stereolithography (SLA). The printing process itself is as simple as melting plastic filaments in an extruder and using them to form layers that create mechanically robust objects (as done in FDM) or using light to selectively polymerize resins step by step to obtain highly intricate and complex forms (as done in SLA) or masked SLA (mSLA).[14] For a starter guide on how to 3D print using FDM technology, see Box 1.

Figure 1. 3D printing process: from concept to reality. 3D printing starts with an idea or a necessity to produce a specific object. For this a digital 3D model is either designed by the user or downloaded from an online database. The file encoding the 3D model needs to be processed in a slicing software, which divides the model into layers and creates instructions that the 3D printer can interpret. Depending on the desired results, different 3D printing technologies can be employed, which use different materials (e.g., SLA uses resins and produces better details; FDM uses polymer filaments and has high mechanical resistant products). Once the product is printed, further processing is possible to achieve a high-quality finish (e.g., smooth surfaces).

Box 1. How to 3D print: A quick start guide

This step-by-step guide allows you to get familiar with the basic steps of planning and performing a successful 3D printing project using FDM, the most common commercial 3D printing technology. This guide is also available as a video (Movie S3, Supporting Information).

Step 1 - Preparations: Before you start off, make sure you have everything you need: a computer, a SD card, a 3D printer, and the printing material. At the time of writing this review, affordable desktop 3D printers capable of performing most printing jobs are the Creality3D Ender-3 V2, the Original Prusa i3 Mk3, and the Anycubic Mega. However, care should be taken to ensure that the 3D printer is adequately sized to print the object as down-scaling or printing subsections is not ideal in most cases, and largely defeats the purpose of 3D printing. As mentioned in the previous sections, the choice of material is crucial. The most commonly used materials for FDM-based 3D printers are polylactic acid (PLA) and polyethylene terephthalate glycol (PETG). Materials for FDM are sold in the form of rolls of filament at a price of approximately €25 per kilogram, but prices vary considerably depending on the quality.

Step 2 - Obtaining a digital 3D model: The process of 3D printing starts with an idea or the need to produce a specific object. This object has to be represented in a digital format precisely defining the shapes and measurements of the object. You can design a 3D model "from scratch" or by modifying a model previously created using open-source or commercial computer-aided design ("CAD") software. TinkerCAD, for instance, is a user-friendly and web-based platform that can be used even by inexperienced users to design virtually any object. More sophisticated and technical programs also exist, which allow for more precise control of the design's parameters and the production of professional models (see also Box 2). The 3D CAD model is then exported in the STL file format (Standard Tessellation Language), which encodes the object's surface geometry but has no information about its color or texture. Alternatively, the CAD and STL files can be directly shared between users, or downloaded from 3D model databases (e.g., https://www.thingiverse.com, also check the "3D-Model Database").

Step 3 - Instructing the 3D printer: Once you have created the STL file, it can be imported into a "slicing" software (e.g., PrusaSlicer or Cura) to generate a set of instructions, called G-code, that can be directly interpreted by the 3D printer.

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The G-code defines the actions that the 3D printer will perform to print the object, including the movements and temperature of the printing head and bed, the rate of extrusion and retraction of the filament, and the printer's speed. At this stage, printing parameters directly affecting the object's structural integrity and aesthetics are defined. Critical parameters are layer height and width, the percentage and pattern of the infill, and the inclusion of support materials and rafts. Besides this, there are a number of options you can explore to optimize printing speed, material consumption, and print quality. Despite this complexity, most slicing software provides an interface that enables easy manipulation of the printing settings and optimized default values.

Step 4 - Printing the object: Finally, the G-code file is delivered to the 3D printer to initialize the printing job. You can either save the G-code file to an SD card or directly upload it to the printer using a free online platform called Octoprint. For this, you additionally need an internet connection, a miniaturized and affordable computer called Raspberry Pi (https://www.raspberrypi.org), and a USB cable to connect the Raspberry Pi to the 3D printer. Setting up a Raspberry Pi and an OctoPrint account requires a one-time additional effort but provides useful functionalities to the user, including remote control over the 3D printer and the ability to live-monitor and record video time-lapse of the printing jobs. We suggest DietPi as a hassle-free operating system for the Raspberry Pi (see https://dietpi.com/docs/install/ for a guide on how to easily install DietPi, and https://octoprint.org for instructions to set up an Octoprint instance). Once the G-code is delivered to the 3D printer, the printing job can be initialized.

Step 5 - Inspection and post-processing: The final object is often ready to use right after printing. Naturally, you should inspect the quality of the printing job. For complex multi-component structures a high-quality print with perfect fit has to be assured for correct and functional assembly. Also, some models contain moving parts printed in one piece (i.e., "print-in-place" models), such as bearings and hinges. These models often require that the user forces the moving parts slightly to "release" them. Moreover, particular objects require post-processing to obtain the final structure. For example, manual removal of support structures or rafts created during printing is necessary. In other cases, for practical and aesthetic reasons, the surface of the object might require a finishing treatment such as sanding and polishing or with chemical solvents such as acetone.

Step 6 - Enjoy 3D printing: 3D printing is a satisfying process: it empowers its users by enabling the materialization of ideas. As you explore the variables involved in 3D printing and their modulation results, you will become more aware of the technology's potentials and limitations, which in turn is a vector for your creativity. 3D printing should be enjoyed, and the best results are achieved through sharing and cooperation. There are several online forums available, where users actively engage with and benefit from the maker community by sharing their designs, advice, and expertise.

Additive manufacturing starts with a 3D model designed from scratch or downloaded from the internet (Figure 1). This is an exciting aspect of the technology, as files can be easily shared online, significantly increasing accessibility. The files are created and visualized in commercial or open-access 3D software (see Box 2). This software usually belongs to the CAD family (Computer-Aided Design), an extensively used software type for drafting and modifying 3D designs.[15] Once the 3D model is ready, it needs to be converted into a ".STL" file, which stands for Standard Tessellation Language. This is often done by the CAD-type software itself but requires additional steps if using non-CAD based software, once the .STL file is obtained, the printing pattern or path needs to be converted into a set of instructions that a 3D printer can interpret (i.e., for FDM, a "G-code"). This step is performed by a "slicer" software, which divides the object into layers and calculates the path that the printer needs to travel to produce them. The "slicing" software can also be commercial or open-access (see also Box 2).

Box 2. Software tools: for 3D design: 3D design software tools

TinkerCAD: free, creation and rendering of 3D models, tutorials and teaching resources, interactive modeler and script based, very accessible even for unexperienced users, browser based (www.tinkercad.com)
OpenSCAD: Free, creation and rendering of 3D models, only script based. It might not be that intuitive to start with, but the short and comprehensible list of commands summarized on the cheat sheet is a great help. (www.openscad.org)
FreeCAD: free, creation and rendering, modeler based (www.freecadweb.org)
Blender: free, 3D model creation, rendering and animation
Fusion360: commercial software tool with free non-commercial subscription for one year (www.autodesk.com/products/fusion-360)
Rhino3D: commercial, free-form 3D modeling, creation, rendering, and animation, handles complex models and point clouds (https://www.rhino3d.com)
3DS Max: commercial, 3D model creation, rendering, and animation (www.autodesk.com/products/3ds-max)
SolidWorks: commercial, 3D model creation and rendering, includes motion and stress analysis tools. (www.solidworks.com)
Free software tools for G-code creation
Slic3r: FDM (www.slic3r.org)
PrusaSlicer: FDM (www.prusa3d.com)
ideaMaker: FDM (www.ideamaker.io)
Ultimaker Cura: FDM (https://ultimaker.com)
PreForm: SLA (https://formlabs.com)
chitubox: mSLA (https://www.chitubox.com)

Once a G-code file is created, the next step is 3D printing. The selection of materials and 3D printing technology will

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depend on various factors, including cost, speed, material characteristics, and needs. This review's scope is limited to commercial and common 3D printers, which use filaments and liquid resins to print with FDM and (m)SLA, respectively. Moreover, other printing technologies use different materials, including metals, ceramics, concrete, and even food.[16] Depending on the machine used and the printing job, the printing process can be lengthy, taking over several days in some instances to complete. Once the object is printed, it can be subjected to post-processing steps.

For example, including support structures in the model is often required to print difficult areas (e.g., bridges and overhangs); these need to be removed manually afterwards or dissolved in water if a water-soluble filament is used (e.g., polyvinyl alcohol). Moreover, some models are printed in pieces that need to be assembled in an extra step. Finally, high-level surface finishes can be achieved by sanding, welding with organic solvents, painting, and polishing.

3. 3D Printing for Microscopy and Microscopy-Related Applications

Concerning microscopy, 3D printing technologies impact two critical aspects: accessibility and rapid prototyping of customized equipment. Along the lines of "Seeing is believing", microscopy plays a crucial role in studying biological processes by providing information far beyond the details perceived by the naked eye. From the early days when Antonie van Leeuwenhoek observed protists and bacteria for the first time,[17] to the modern super-resolution solutions capable of resolving images below the light diffraction limit (300 nm), microscopes represent crucial tools in biology research.[18]

Microscopy technology today is currently still limited in its application, often requiring high investment and specialized training. This puts researchers with limited funds at a disadvantage, as access to sophisticated imaging approaches are only available to researchers from wealthy countries. This has given rise to a new movement in microfabrication and microscopy to create open access and inexpensive technology accessible to a broader audience. In recent years, 3D printing-based solutions drastically increased the capability to design and fabricate scientific instruments, reducing fabrication time, cost, and structural limitations. More importantly, low-cost and highly sophisticated research tools are now accessible to a broader range of researchers, allowing a worldwide audience to benefit from better technology without relying on commercial solutions. 3D printing also facilitates iterative design-based approaches that would be difficult, if not impossible otherwise. Additionally, these benefits are available to the general public, as commercial 3D printers are now more accessible than ever. This aspect is particularly important because it enables "citizen scientists", empowering existing research avenues by increasing research output via crowdsourcing, benefitting society. Many examples of researchers taking advantage of the easy accessibility of 3D printing technology exist (see also Movie S1, Supporting Information). For example, inexpensive microscopes for education are being produced, such as the FlyPi.[19] Also, the highly complex robotic microscope OpenFlexure[1] represents a clear desire
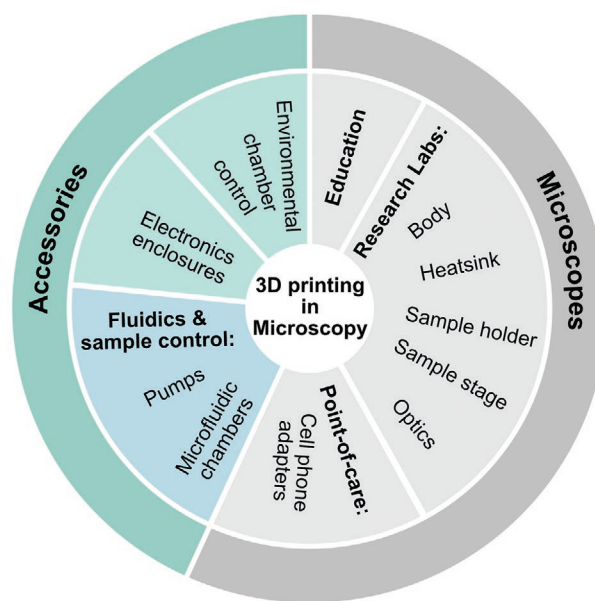

Figure 2. Application fields of 3D printing for optical microscopy in life sciences. Additive manufacturing is currently used in many open-source projects in microscope modular designs. Printing whole microscope bodies at relatively low prices is now possible, except for a few parts. Additionally, 3D printing is used for sample control, such as microfluidics systems and sample holders.

to democratize this technology. On the other hand, additive manufacturing is rapidly becoming the tool of choice in microscopy technology development, as 3D printing technology such as FDM allows rapid prototyping with an unprecedented level of freedom. This freedom in design paired with the unmatched speed in generating prototype components creates a perfect combination for innovating new cutting-edge technology where highly complex components can be fabricated and iterated upon with relative ease (**Figure 2**). These characteristics enable systems such as miCube[20] and the UC2,[21] where further customization is possible due to their modular design. The microscope parts that can be built vary with the technology and materials available. In the literature, whole microscope bodies have been printed, including the base,[20–22] the body,[20,22,23] holders for the filters,[22,24] objectives,[25] coverslips,[21] pin-holes,[21,23] and heat sinks.[25] Microscope chambers[26] and controller mounts[27] have also been implemented, allowing a high degree of customization to researchers adopting this technology.

3D printing is also used to print tools that allow for more complex microscopy solutions. Sample manipulation is an important aspect of this process, including sample holders, sample surveying, and microfluidics systems (see also Movie S2, Supporting Information). Sample holders are in direct contact with the sample, requiring biocompatible materials that are readily available or made biocompatible by post-processing. Examples include cell grid holders[28] and incubation chambers.[29] Microfluidics can be used to control the movement of small liquid and particle volumes in a network of interconnected microchannels. Microfluidics is useful to dispense, mix, separate, and detect different reagents into a sample, allowing a high degree of manipulation using a system

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of automatic miniature pumps and Lab-on-Chip devices. Additionally, pump systems have been fully automatized to allow sample manipulation for downstream microscopy applications such as fixation and immunofluorescence.[30,31]

4. 3D Printed Microscopy Projects

Microscopes are usually highly intricate machines composed of a multitude of parts with varying degrees of complexity. These parts have been traditionally built using standard micro- and macro-fabrication methods that are expensive and, in some cases, restrictive. Implementing 3D printing approaches in the rapid fabrication and prototyping of optomechanical components allows new microscopy approaches, particularly when the designs incorporate methods to sense, process, and act automatically via computer-based controllers such as Arduino or Raspberry Pi. It has been reported that 3D printing can reduce microscope parts price to between 50% and 90%, depending on the component.[32] The performance of the 3D printed parts (kinematic mounts, translation stages, and integrating spheres) were directly compared to commercial counterparts to assess their precision and performance. One of the tradeoffs that must be accepted is the printed part's limited physical integrity, comparable to low-end commercial alternatives.[32] Nevertheless, smartly designed and highly tailored components such as a monolithic 3D printed flexure translation stage have been realized with this approach. This stage was capable of submicron-scale motion with remarkably low drift and minimal post-processing.[33] Micromanipulators and probe position systems have also been 3D printed and tested using flexible materials with substantial price reductions compared to commercial options.[34] Even tunable objectives have been realized by 3D printing poly (methyl methacrylate) (PMMA) singlets containing miniaturized electrowetted lenses for electronic focusing within a 3D-printed housing.[35] While the fabrication of optically active elements usually requires nanoscale precision far beyond the capability of the 3D-printing techniques featured in this review, the implementation of near-refractive index-matched media allows realizing a phase mask based on a 3D-printed mold with micrometer topography.[36] By employing post-fabrication surface treatment and polishing 3D printed optically transparent prisms for surface plasmon sensing have been realized.[37] Beyond this, low-cost, compact, and high-performance illumination systems have been developed employing 3D printed components, such as the NicoLase project.[27] NicoLase is an open-source diode laser combiner, fiber launch, and illumination sequence controller for fluorescent microscopy and super-resolution applications that successfully competes with the performance of commercial systems at half of the costs.

The increase in mobile phone usage backed by powerful cameras and increasing computational output has given rise to mobile microscopy. Although not as powerful as high-end dedicated cameras, it is still possible to use cellphone cameras to capture microscopy data after adapting hardware and software for this purpose. Mobile smartphones have achieved enough computational power that acquisition and processing are now possible in the same device.[38] This practice can be beneficial in fieldwork where conditions are not ideal for laboratory

equipment. Projects such as cellSTORM have shown that it is possible to achieve SMLM using consumer mobile phones and achieve optical resolutions higher than 80 nm.[38] The project also benefits from using a trained image-to-image generative adversarial network (GAN) to reconstruct video sequences under suboptimal conditions, improving signal-to-noise ratio by compensating noise and compression artifacts. As the high-performance scientific camera is usually one of the most expensive components of a microscope, exchanging it for an industrial-grade or even a mobile phone camera significantly reduces the overall costs of the system, even more so by integrating this approach into a 3D-printed microscope.[39] More importantly, the widespread use of mobile phones worldwide provides easy access to technology capable of acquiring images, particularly in developing countries.[40] The FPscope project has pursued this approach, creating a system capable of high-resolution imaging using variably illuminated, low-resolution intensity images in Fourier space, called Fourier ptychographic microscopy.[41] Here, the mobile phone lens is used in a reversed manner where the mobile phone lens projects the magnified image to the detector. The μSmartScope is another example of a 3D printing adaptor fitted into a wide range of mobile smartphones.[42] In addition, the motorized stage is fully automated and controllable by the smartphone and is capable of autonomous image acquisition. Especially for particular tasks where limited camera performance and computation power are sufficient, smartphones are used for microscopy applications to enable point-of-care and field diagnostics. These kinds of applications are, for example, DNA fluorescence spectroscopy for the readout of fluorescence-based biological assays to detect specific nucleic acid sequences reaching the point of detecting single-base mutations.[43] Other DNA-based applications include a surface-heated droplet PCR system to detect *Escherichia coli* that employs wire-guided droplet manipulation to guide a droplet over three different heating chambers.[44] Following product amplification, end-point detection is achieved using the smartphone-based fluorescence microscope. Automated cell identification has also been achieved using a 3D printed shearing digital holographic microscope for fieldwork.[45] It uses a common path shearing interferometer for automatic cell identification via a CMOS or a mobile phone camera and the addition of laser illumination and a commercial objective. Another holographic lens-less smartphone-based microscope has been designed for holographic micro-object imaging of various samples using a CMOS camera chip and controlled by a Raspberry Pi.[23] Further examples of smartphone-based projects differentiate between white blood cells with acridine orange staining using a miniature achromatic microscope,[46] identify pathogenic bacteria using a DNA-based FISH assay,[47] and image and identify malaria parasites with a ball lens objective capable of high-resolution, bright field imaging of Plasmodium parasites in blood smears for field diagnostics.[48] One of the most exciting applications of 3D printing in microscopy is the generation of fully functioning microscopes with few non-3D printed components. Beyond the iterative design aspect, many 3D printed microscopes are open-source projects that present a unique opportunity to provide access to novel ground-breaking designs to a wide range of researchers bypassing geographical and economical limitations.
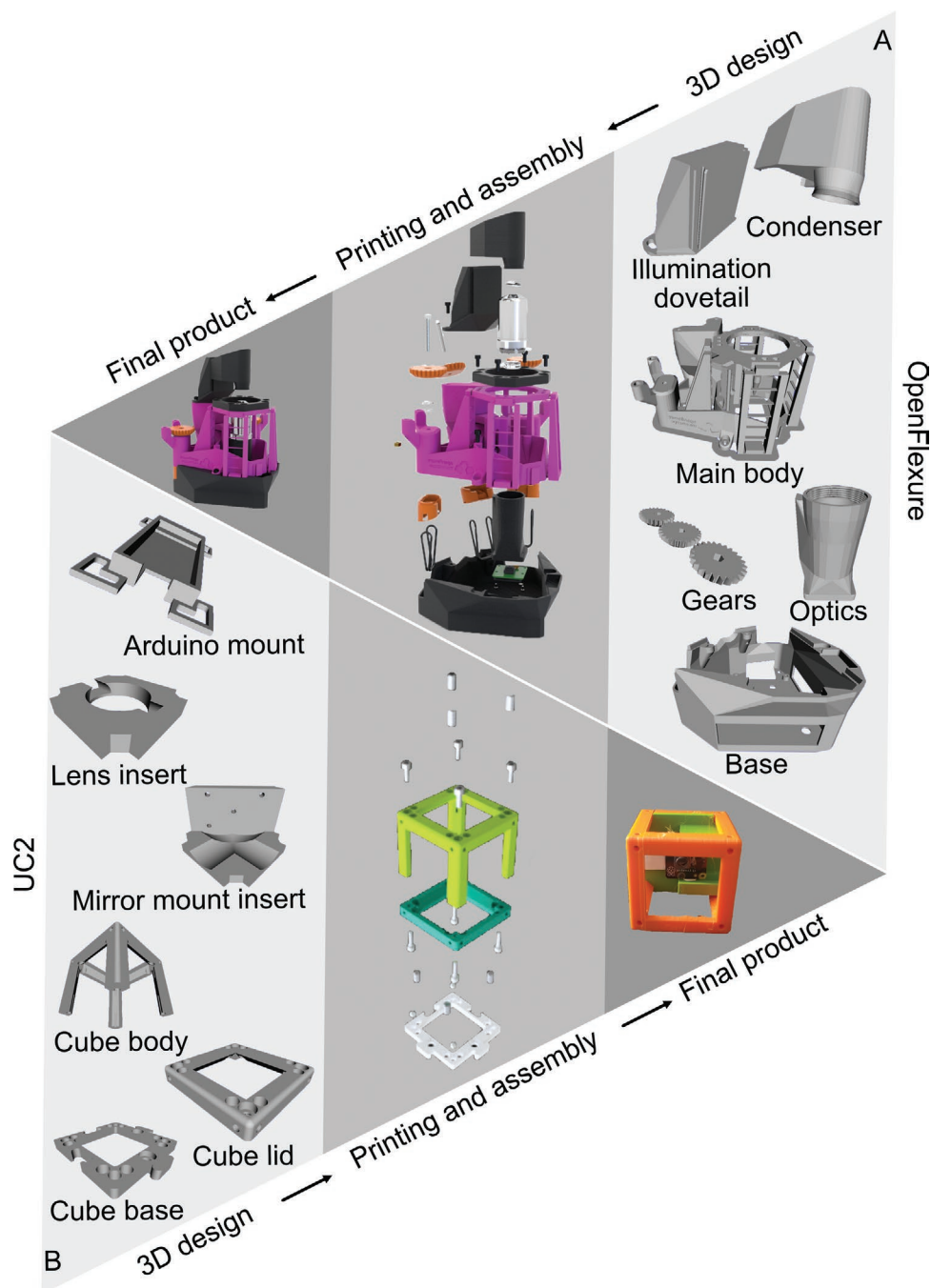
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Figure 3. 3D printed microscope parts. Microscope parts can be 3D printed and can be assembled to create a fully working microscope. A) The Open-Flexure project is an example of an almost fully 3D-printable microscope [https://bit.ly/2Yu8oQ4]. Adapted under the terms of the CC-BY license.[1] Copyright 2020, The Authors, published by Optica Publishing Group. B) The UC2 project is based on cubic structures that integrate inserts to allow microscopy techniques such as SIM. Certain parts cannot be 3D printed and are sourced independently (e.g., lenses and electronics) [https://bit.ly/2YnS83e]. Adapted under the terms of the CC-BY license.[2] Copyright 2020, The Authors, published by Springer Nature Group.

Fully functioning 3D printed microscopes often present a modular design, allowing us to add or remove components according to the needs of a particular experiment. For example, the μCube project uses cubes to create a framework for a 3D printable microscope using the parametric design of modular mounts.[49] Additionally, it facilitates the alteration of the original design, allowing the generation of new concepts.

One of the most prominent examples of open-source microscopy projects is the OpenFlexure microscope (**Figure 3**A).[1] OpenFlexure is a fully automated laboratory-grade microscope capable of using motorized sample positioning and focus control. Additionally, it is highly customizable, allowing trans- and epi-illumination, polarization contrast, and epifluorescence imaging. It also uses high-end objectives and employs

an 8MP CMOS sensor Raspberry Pi camera (V2) calibrated to use custom optics.[50] The OpenFlexure microscope is controlled using the OpenFlexure software stack that is both cross-platform and language-independent. Control is split between a client and a server application interfaces with a web API using a W3C Web of Things standard. This characteristic provides a modern interface, multi-language support, minimizes code duplication, allows multiple microscopes to be controlled by a single computer, and integrates research experiments, users, and equipment.[51] The OpenFlexure project was co-developed between the University of Bath and the Tanzanian company STICLab. Co-development of a project such as this shows the potential of open-source 3D printing projects where geographical boundaries no longer limit scientists from low-income countries to access better resources, improving their research freedom.[13] The system also demonstrates that a clever design of intricate 3D printed devices can achieve excellent mechanical precision and stability. This even allows for performing super-resolution imaging by combining the OpenFlexure with super-resolution radial fluctuations analysis (SRRF).[52,53]

Another example of a 3D printed microscope with super-resolution capacity is the Chea(i)p. This self-contained super-resolution microscope uses a commercial objective and a mobile phone and costs less than €800.[54] The mobile phone does the acquisition, processing, hardware control, and photonic-chip illumination in this case. Impressively, it can reach resolutions of 100 nm with SMLM and live super-resolution with super-resolution radial fluctuations analysis (SRRF). Furthermore, the waveguide-PAINT system allows a highly uniform 100×2000 μm^2 area evanescent field for TIRF illumination. The system was developed as a stable, low-cost microscope with a 3D-printable chip holder to facilitate alignment and imaging. The waveguide-PAINT can image multiple whole cells or whole origami structures such as microtubules in COS-7 cells in a single field of view.[55]

3D printing has also been adapted to selective plane illumination microscopy (SPIM). SPIM, also known as light-sheet microscopy, is extremely useful for volumetric imaging of large samples as optical sectioning is achieved by employing a sheet of light as illumination. The OpenSPIM project is an open-access platform that allows new users to build a basic SPIM microscope step-by-step.[25] Beyond applications in education and scientific outreach,[56] the system can be upgraded and adapted for specific requirements and budgets. Although considered a challenging endeavor, the OpenSPIM was adapted and used to image the organism *Maritigrella crozieri* using two-color laser illumination to detect two probes simultaneously.[57] Another SPIM 3D printed system is the Flamingo, which offers the possibility of a highly customizable microscope that is adaptable to individual needs while portable (https://involv3d.org). The main idea of this project is to provide access to SPIM systems to researchers worldwide in the form of travelling shareable instruments.

The CellScope project uses a programmable domed LED array, enabling simultaneous multi-contrast imaging in bright-field, darkfield, and phase imaging modes.[58] It works by scanning through illumination angles that capture light field datasets. These datasets recover 3D intensity and phase images without hardware alterations. It can also refocus digitally to achieve either 3D imaging or software-based correction, bypassing the necessity of precise mechanical focusing during acquisition.

Great examples of reducing costs and reuse of common laboratory parts are found in projects such as are the **OPN Scope** that aims to lower the costs for fluorescence microscopy[59] and the FlyPi, which uses a 3D-printed mainframe, a Raspberry Pi computer, a high-definition camera system and Arduino-based optical and thermal control circuits for €200 or less.[19] The system was tested in experiments involving behavioral tracking in *Caenorhabditis elegans* as well as Optogenetics and Thermogenetics in Drosophila and *C. elegans*. The miCube open-microscopy framework is another system of this family.[20] It has been used to visualize dCas9 in vivo target search, as it is capable of single-molecule microscopy with high spatiotemporal resolution. The Microscopi project aims to democratize microscopy with portable, low-cost, 3D printed and self-built systems capable of multimodal imaging (bright field, dark field, pseudo-phase, and fluorescent microscopy).[60] It uses an automated XYZt imaging system controlled by a tablet or smartphone using a simple GUI. The PUMA microscope adds the possibility of direct visual observation with an augmented reality display.[61]

Lastly, the UC2 (You. See. Too.) project is a low-cost, highly versatile, and customizable 3D printed microscope with a modular design toolbox.[21] The system is fully accessible online and uses many standard off-the-shelf optics and electronic components fitted in 3D printed cubes (Figure 3B). It has been used to acquire macrophage cell differentiation data, as well as apoptosis and proliferation enclosed in an integrated incubator in one of the modules and by minimizing axial drift with an automated focusing system.

5. Sample Manipulation, Microscopy, and 3D Printing

3D printing applications for sample manipulation are highly versatile and powerful tools. The field of microfluidics is an exciting target for applying additive manufacturing, allowing us to downscale biochemical applications to create portable and nano-scale versions of a testing laboratory. While the field was initially based on molding and replica molding fabrication, today, 3D printing allows for more complex geometries and designs. With this approach, the production of moving parts is possible, including miniaturized pumps and valves that enable accurate fluidics control, as well as sensors that allow the detection of micro-changes in the environment (**Figure 4**). Additionally, the variety of 3D printing materials available will enable researchers to customize the parts to suit specific needs.[62] Functional components such as valves are divided into manual and pneumatic, with the former requiring manual control and the latter requiring an energy source. Several examples of valves exist in the literature, ranging in complexity. Simple manual valves consist of an enclosed valve that allows for flow when the inside and outside channels are aligned in the opened position.[62,63] Other more complex valves, such as pneumatic ones, can be printed in arrays to mimic circuits[63] or quake valves.[64] Another method to control microfluidics is using pumps. Syringe pumps are simple and can deliver precise volumes of liquids for various research needs (e.g., delivery of drugs to samples during live imaging).
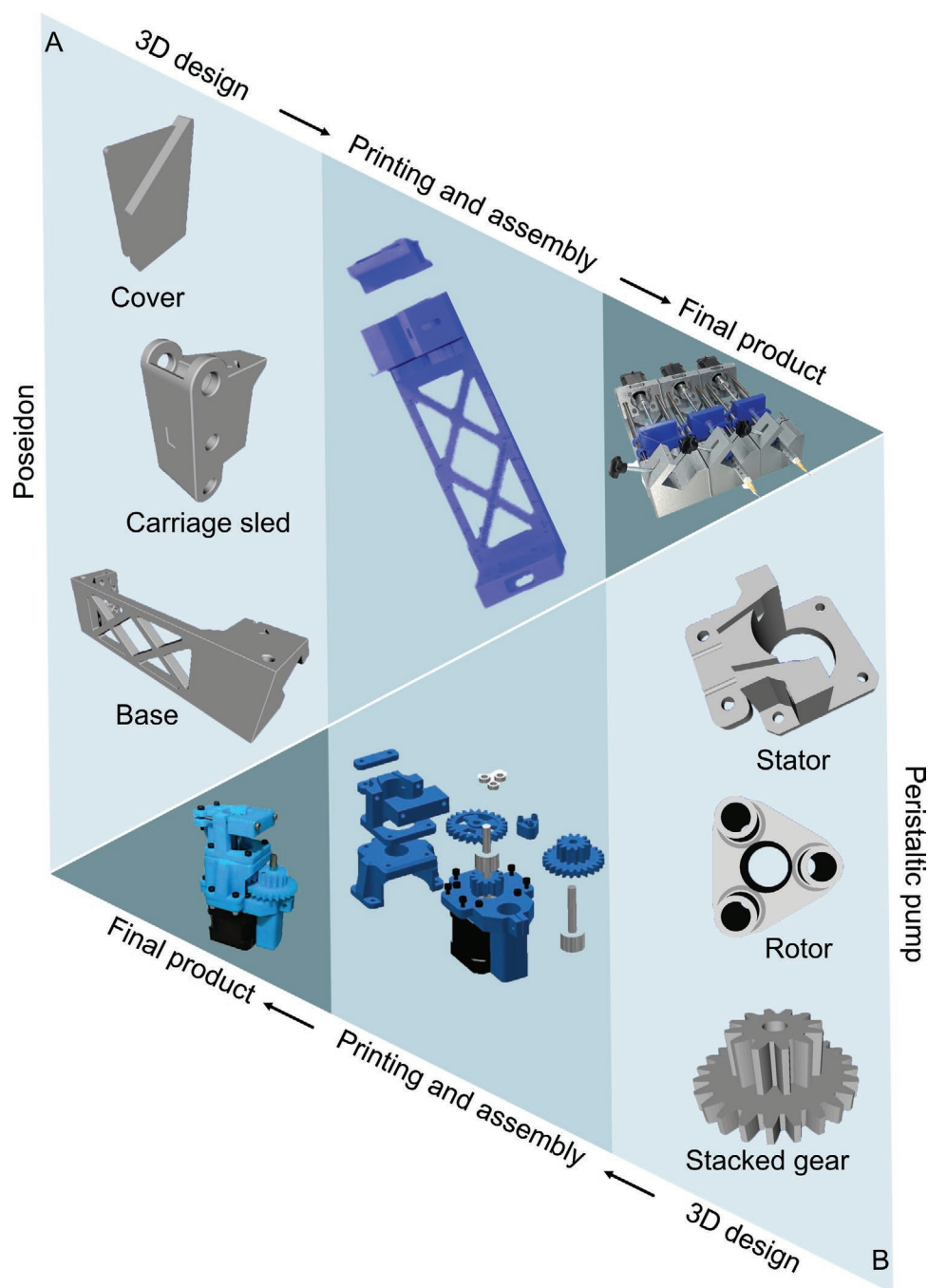
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Figure 4. 3D printed parts for fluidic control. A) "Poseidon": a syringe pump-based system that supports multiple pump arrays [https://bit.ly/3BlGuoe]. Adapted under the terms of the CC-BY license.[31] Copyright 2020, The Authors, published by Springer Nature Group. B) Peristaltic pump system [https://bit.ly/3mAP1O1]. Adapted under the terms of the CC-BY license.[69] Copyright 2020, The Authors, published by Springer Nature Group. Both approaches require an external power source and can be controlled using an electronic board.

These pumps use a motor that drives the precise movement of the syringe plunger, resulting in accurate fluid volume control. Syringe systems can be paired in multi-pump arrays that facilitate multiple fluids to be pushed in and out of samples. While commercial systems can be expensive, 3D printed options are available that are both highly efficient and highly modular, allowing the addition of more syringe pumps easily (Figure 4A).[30,31] The highly modular lego-and-3D-printed system NanoJ-Fluidics (aka "Pumpy") is capable of managing up to 128 syringe pumps simultaneously.[30] These systems are controlled by Arduino controllers, allowing for precise fluid volume manipulation. Direct pump control via G-code can be easily realized by repurposing motors and controllers of a 3D printer.[65]

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Similarly to syringe pumps, fully 3D printed pumps are capable of inducing flow.[66] The most common pump is based on peristaltic pumps, usually consisting of three or more valves along the flow channel. 3D printed peristaltic pumps based on planetary gear concepts are practical and can be printed using common FDM or SLA 3D printers (Figure 4B).[67] These pumps can be employed in microfluidic automation and printed using designs that do not require posterior assembly (i.e., "print-in-place").[68] Furthermore, they can also be operated with Arduino controllers, allowing for custom flow profiles for handling precise liquid volumes.[69,70] 3D printed pumps are inexpensive to print, with prices as low as €38 per channel.[70] Peristaltic pumps 3D printed using SLA can present durability issues. However, using thermal initiators in the liquid resin and post-processive baking of the component has significantly improved durability.[71] Some of these pneumatic pumps require complex ancillary control tubing. However, multiplexers allow for control over multiple valves employing only a few control channels, thus facilitating the upscaling of designs and adaptation into highly compact and complex microfluidic systems.[72] Microfluidic chips can work as sensors, detecting physical and chemical changes inside chambers by measuring volumes on the picoliter scale.[73–76]

Microfluidics devices are currently fully 3D printable[77] with the caveat of not providing transparency.[72] Printing glass-like transparent material is still problematic for 3D printing. Although examples of 3D printed prisms[37] and microfluidic devices with optical readouts[12] can be found, optimal optical properties are still challenging to obtain. To circumvent this issue, certain parts, such as valves, can be 3D printed and then combined with transparent materials such as glass or poly-dimethylsiloxane (PDMS). For example, 3D-printed scaffolds made of acrylonitrile butadiene styrene (ABS) or water-soluble polyvinyl alcohol (PVA) filaments have been used here. A mold is then used to cover the scaffolds with PDMS. As soon as the PDMS has hardened, water dissolves the PVA filaments without leaving residues, while ABS can be dissolved by acetone. This approach presents the advantage of printing directly onto the coverslips while also providing transparency, allowing excellent applicability for imaging.[78,79] Besides lowering costs, one of the advantages of using 3D printing compared to other microfabrication methods is the more complex channel designs that can be achieved, such as serpentine flow channels with cross-sectional areas[77] and the compatibility with additional microfabrication approaches like micropatterning.[80] Additionally, bonding printed channels to transparent poly (methyl methacrylate) (PMMA) sheets makes it possible to produce highly complex arrays, such as straight, spiral, serpentine, curvilinear, and contraction-expansion.[81] In some cases, designs such as T-shape pillars are impossible to obtain via other traditional fabrication methods in a single demolding step, but only by 3D printers.[82]

Furthermore, 3D printed microfluidic chips have been used to monitor pathogenic microorganisms. For example, using an ABS polymer and FDM 3D printing, a chip allowing bacterial culturing, DNA isolation, PCR and posterior detection using gold nanoparticle (AuNP) probes as an indicator of *Staphylococcus aureus* (MRSA) was devised.[83] A colorimetric assay based on the interaction between the MRSA mecA gene and AuNP probes was used to confirm the bacterium's presence in the samples. An important limitation of the generation of effective microfluidic chambers on these chips is the dimension of the channels.[81] The Miicraft printer was designed with this problem in mind and can print complete microfluidic chambers for lower prices compared to other methods.[84] This system was used to create an in vitro model of the circulatory system using a cardiac-like on-chip pumping system. This was done using four pumps and passive check valves to mimic the four heart chambers and valves. The process was later validated by emulating normal human left ventricular and arterial pressure profiles.

6. Biological Cages and Direct Sample Manipulation

Sample holders and biological cages that are in direct contact with living organisms are critical imaging components. 3D printed parts used for these applications require biocompatible materials that do not alter the physiology of the cells. While many strategies to ensure biocompatibility exist, generally using ethanol washes with epoxy resin appears to provide the best results, even when using transparent materials.[85] Depending on the sample holder's nature or biological cage, autoclavable material might be helpful for prior- and post-sterilization during experiments. Structures with these characteristics require printing with advanced plastics that provide high steam, temperature, and physical resistance.[86,87] More information on material properties and their biocompatibility can be found in Table 2. An advantage of using customized sample holders and biological cages is the possibility of tailoring them to fit different imaging platforms. Microscope parts are often incompatible between different manufacturers or machines. This limits, for example, cross-instrument compatibility in cutting-edge microscopy facilities, where it is common to find many different microscope types. To solve this, the UniverSlide project created a multi-stage sample biological chamber that can act as a holder or cage for specimens, allowing for the growth of living tissue and easy adaptation between microscopy systems.[88] This versatile sample chamber was 3D-printed using SLA and a biocompatible HTM140 resin from Envisiontech (**Figure 5**A). The authors agreed that the system could be adapted to use standard resins since glass and PDMS are the only materials directly contacting the sample.

UniverSlide was devised to have the dimensions of a regular microscope glass slide (e.g., 26×76 mm^2) and uses five main parts that include the 3D printed chamber frame, a bottom coverslip, an agarose pad, a PDMS seal, and a 3D printed lid with a glass slide (Figure 5A).[88] The sample chamber can then be filled with a cell culturing medium for microscopy applications in unicellular and multicellular samples. Also, it is compatible with live and fixed samples.

This type of sample chamber can also be expanded to aid experimental procedures. For example, a low-cost cell growth chamber capable of electrical or chemical stimulation of the sample has been devised.[89] Electrical stimulation in mammalian cell cultures is used to assess physiological mechanisms, generally in neuronal cells and myocytes. They are also used to time-resolve intracellular calcium concentrations as a direct result of inducing membrane depolarization.

Direct sample manipulation is also possible, for example, using tension and other mechanical stimuli to determine tissue
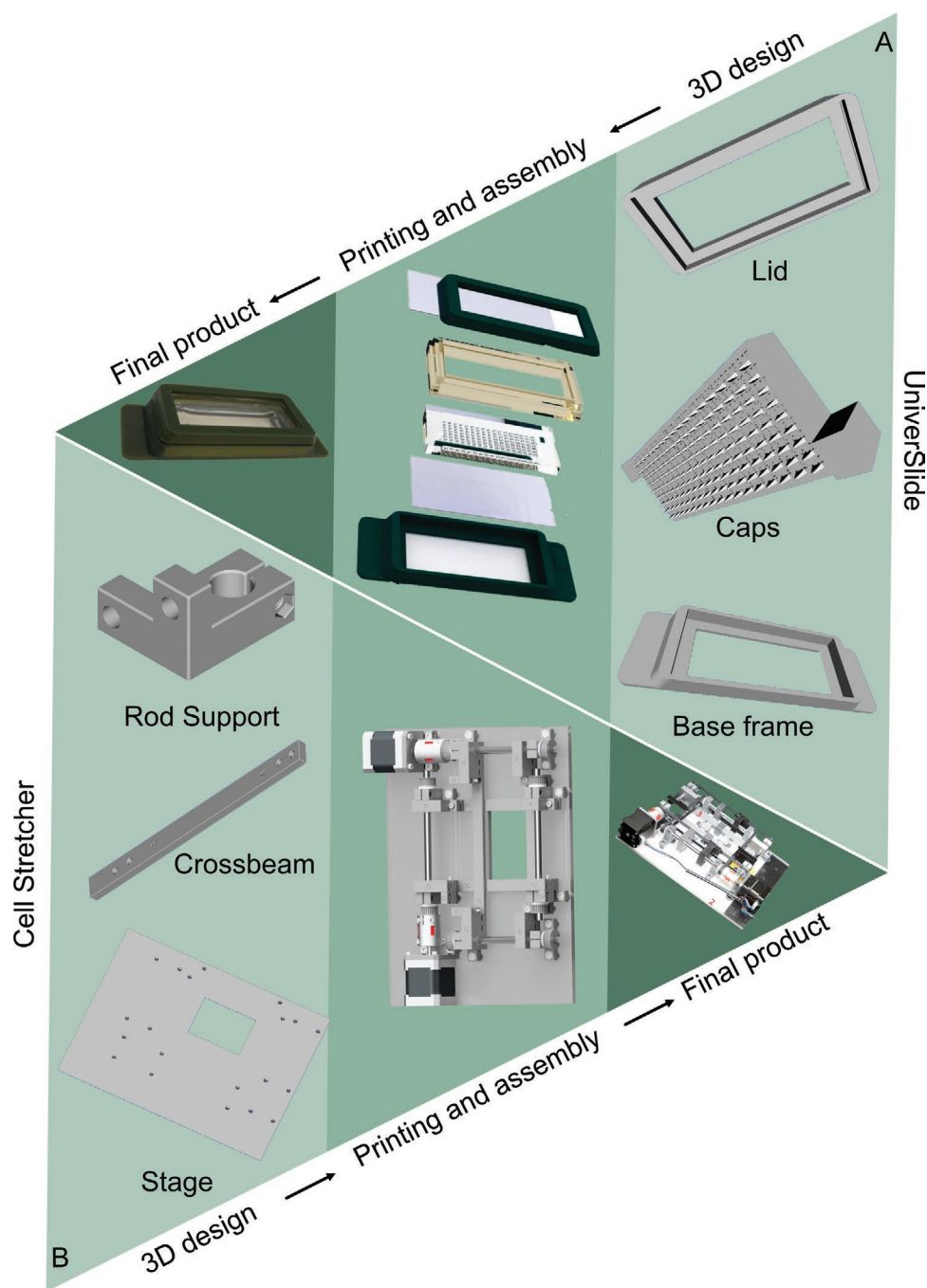
Figure 5. 3D printed parts for sample holders and manipulation. A) The UniverSlide is an all-in-one 3D printed microscopy chamber for multidimensional imaging [https://bit.ly/3Fm7RAS]. Adapted under the terms of the CC-BY license.[88] Copyright 2017, The Authors, published by Springer Nature Group. B) Cell stretcher used to assess tensile properties with controlled cell stretching. This open-source system is directly mounted on the microscope stage, allowing fluorescent live cell imaging [https://bit.ly/3AeYqiN]. Adapted under the terms of the CC-BY license.[91] Copyright 2019, The Authors, published by Elsevier.

properties. This process is achieved using devices that stretch the cell/tissue sample in a controlled manner and measure mechanical properties while monitoring cellular changes using fluorescence microscopy. Commercial cell stretchers are available[90] but are often expensive and hard to customize. The 3D-printed Open source Biaxial Stretcher (OBS) was developed for this reason, improving accessibility to researchers.[91] Additionally, it is compatible with upright and inverted fluorescence microscopes and can perform up to 4.5 cm XY-stretches using an electronic controller (Figure 5B). A second example for a direct sample manipulator is the 3D printed and motorized micropositioning device which allows for directing a needle, probe, or syringe in x, y, and z (https://open-labware.net/projects/micromanipulator/).

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7. 3D Printing Resources and Technology in Microscopy Applications

This review aims to enable researchers unfamiliar with 3D printing to implement this approach for their microscopy projects by providing a comprehensive guide of available resources and technologies, including a quick start guide (see Box 1).

7.1. Databases for 3D Parts

3D printing databases exist for research (e.g., NIH Exchange: a database of 3D printed parts) and more general applications (e.g., Thingiverse), providing 3D printing enthusiasts with the means to obtain complete 3D models. However, a comprehensive database focused on microscopy projects does not exist. This represents a substantial obstacle for newcomers, as information is not centralized and can be challenging to locate because it relies on previous literature knowledge. Therefore, we compiled a 3D printing database for microscopy applications which can be found as Supporting Information.

7.2. Commonly Used 3D Printing Technologies

Additive manufacturing comprises a variety of different technologies. Although the technology is widely known and highly advertised, researchers unfamiliar with 3D printing technology still struggle to fully realize its potential beyond mere curiosity. 3D printing technology has seen a sharp increase in accessibility as the technology becomes less expensive, with many commercial options offering basic printers for less than €100.[14] From these widely available printers, FDM and SLA stand out as the most straightforward and inexpensive options for a beginner.[92]

FDM is perhaps the most popular and cost-efficient of all 3D printing technologies. It is versatile and flexible, with many different materials available to suit specific needs. The most common materials used in FDM are thermoplastics, but composites of thermoplastics and ceramics or metal powders are also available. During the 3D printing process, the plastic material is extruded through a heated nozzle along a predefined path and deposited layer by layer to materialize the design. The printer consists of a platform, the print bed where the layers will be deposited in a semi-solid state, a print head composed of a heating block and a nozzle, electric motors that move the print head, and the filament spool holder. Once the G-code of the design is loaded into the printer, a three-axis system controls the print head, moving in the x–y axis to deposit a layer with paths covering the shape of the initial slice of the 3D design. After each layer is deposited, the print head or print bed resets its position along the z-axis according to the layer thickness chosen, and the next layer is deposited using x–y axis movements. This process is iterated until the final 3D model is recreated. Due to its simplicity and fast turnaround, FDM excels at rapid prototyping. However, the printed structure's quality depends on a multitude of factors, including the material used.

Furthermore, other elements besides the materials used contribute to the integrity and properties of the prints (**Figure 6**A). Several studies have experimented with path-planning and part-orientation to alter the anisotropic mechanical properties of the 3D printed parts.[93] The quality of the layer-to-layer binding is also crucial as voids forming between the layers reduce the object's strength. An approach to improving these properties is developing composite materials exhibiting higher mechanical, electrical, and thermal properties. These materials are produced by combining the base polymer with fillers. Another approach to increase structural integrity that is highly discussed in the 3D printing community is infill modulation. Different infill densities can create objects ranging from completely hollow (0% infill) to completely solid (100% infill) (Figure 6B). Significantly, this choice influences the weight of the final object and the printing time substantially. Furthermore, the geometry of the infill pattern chosen is also important. For example, a study found that the "rectilinear" pattern resulted in the highest tensile strength than the other patterns evaluated.[94] However, while "rectilinear" excel at resisting forces applied in the direction of the pattern's lines, it is fragile against forces applied in other directions. Thus, a better choice might be the "cubic" or "honeycomb" patterns, which are less resistant than the "rectilinear" to forces applied in a specific direction but stronger in the others (Figure 6C). Despite the many advantages of FDM compared to other 3D printing modalities, this approach's nature entails certain caveats. FDM is prone to defects and printing artifacts that impact the printed object's aesthetics and practical applications. For example, objects containing overhangs with more than 45° require the use of supports to be printed correctly (**Figure 7**A). These are often removed manually but can also be removed chemically if special dissolvable materials are used to produce them, the most common being the water-soluble PVA. The support removal process results in rough surfaces that require sanding or polishing to be smoothened.[95]

Several printing artifacts can result from a multitude of factors (Figure 7B). "Warping" is a curling deformation often accompanied by a partial or complete detachment of the object from the printing bed (Figure 7B-i). It can result in catastrophic failure if the deformation causes the object to intercept the nozzle's path. Warping occurs as a result of the materials' expansion coefficient. When the material is melted before extrusion, it first expands and then shrinks slightly when it cools down. Thus, warping is more common in materials with higher melting temperatures (e.g., ABS). It can be minimized by controlling the printing environment, for example, using heated printing beds and enclosed build chambers. Conversely, a high bed temperature and insufficient cooling can result in a first layer that is slightly larger than the subsequent layers, an effect known as "elephant's foot" (Figure 7B-ii). This deformation is especially problematic in objects that require a precisely defined shape, such as a part intended to fit a tight slot. Elephant's foot is more frequent in larger prints, in which the weight of the object presses down on the partially-cooled first layers. A nozzle positioned too close to the printing bed may also generate this effect by forcing material extrusion beyond the predicted line width. Thus, "elephant's foot" is typically solved by adjusting the printer's settings and environment. Another solution for
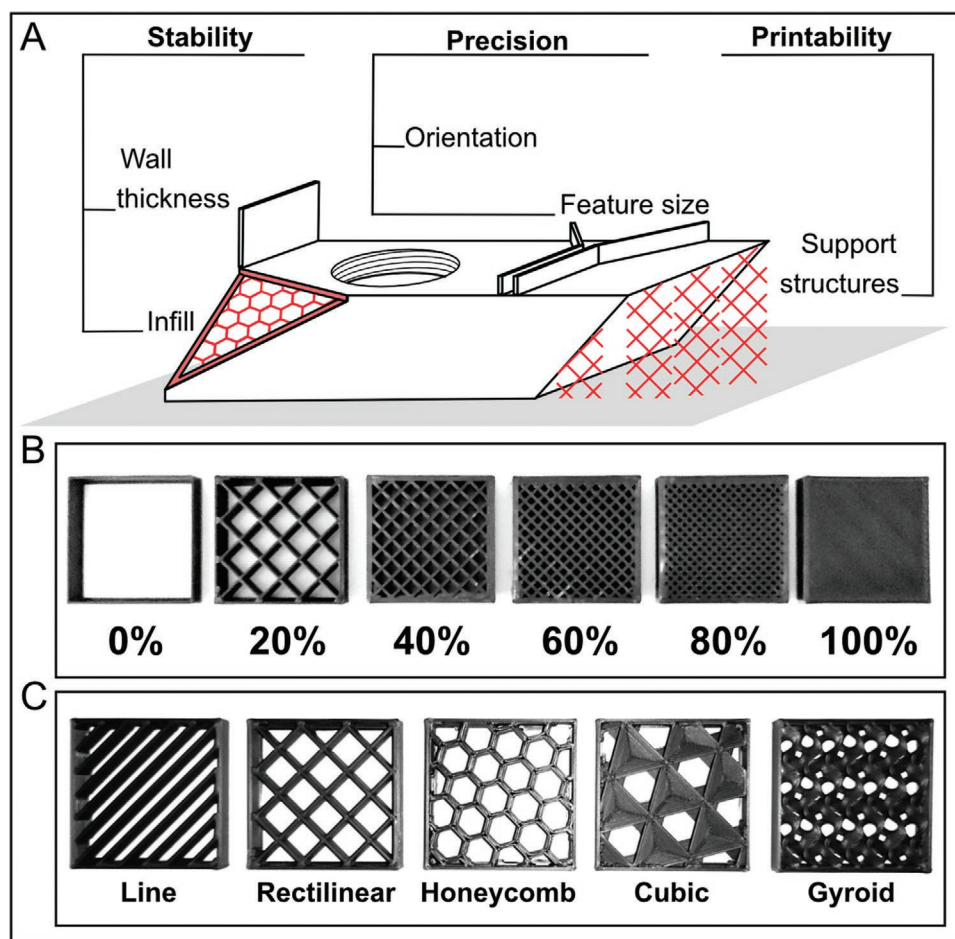
Figure 6. 3D design principles to ensure mechanical stability, precision, and printability. A) The overall mechanical strength of a 3D-printed object is mainly influenced by the thickness of its walls and the properties of the infill (e.g., density and pattern). A high printing precision can be achieved by optimizing the print orientation while the minimum feature size achievable has to be taken into account. Overhangs and bridges might require the implementation of support structures to be printable. B) Examples of different infill densities using the "rectilinear" pattern. A higher infill density contributes to the object's structural integrity but increases the printing time and material consumption. C) Examples of different infill patterns printed with a density of 20%. The infill pattern geometry influences the mechanical resistance of the object.

warping and "elephant's foot" is printing a raft, an additional and wider first layer meant to be damaged in place of the original first layer (Figure 7B-iii). Similar to supports, rafts need to be removed after the printing job is finished, resulting in rough surfaces that might require post-processing.

Another common printing artifact is "stringing" or "oozing", which happens when a material is extruded while the nozzle is moving to a new location (Figure 7B-iv). Consequently, thin strings of plastic are left behind. Possible solutions to overcome "stringing" are increasing the speed at which the extruder moves to reduce the time when material is extruded between movements or increasing the speed and length of "retraction", where the filament is pulled back before moving the nozzle in a new position. In contrast, increasing the movement speed past the capabilities of the printer's motors might result in material extrusion before the nozzle reaches the desired position. Thus, the layer(s) printed will be misaligned with the previous layers, causing a catastrophic artifact named "layer shifting" (Figure 7B-v). Tuning a 3D printer to avoid these and other printing artifacts is now more accessible due to the availability

of several 3D models that can be used to optimize specific printing settings. Some tuning models are directed towards tuning a particular aspect, while others are "all-in-one" models that combine multiple features of 3D printing (Figure 7B-vi).

Stereolithography (SLA) is the oldest form of 3D printing.[96] SLA is a highly versatile and accurate form of 3D printing. Although SLA is more expensive than FDM, the intricate details are fairly superior to the FDM. Instead of using thermoplastics like FDM methods, it uses thermoset liquids—in liquid resins—cured by UV light. Here, UV light selectively illuminates a small liquid resin area, triggering initiators and photo-polymerizing it via radical polymerization, an exothermic process.[97] The polymerization triggered by these initiators creates covalent bonds between the liquid resin monomers. Two transition states occur during this light-based curing process: gelation, where the material transitions from liquid to rubber, increasing its viscosity, and vitrification, where the rubber-like material transitions into a solid resin.[97] This process is done in layers, but due to resin materials, the material's physical properties, such as tensile strength and
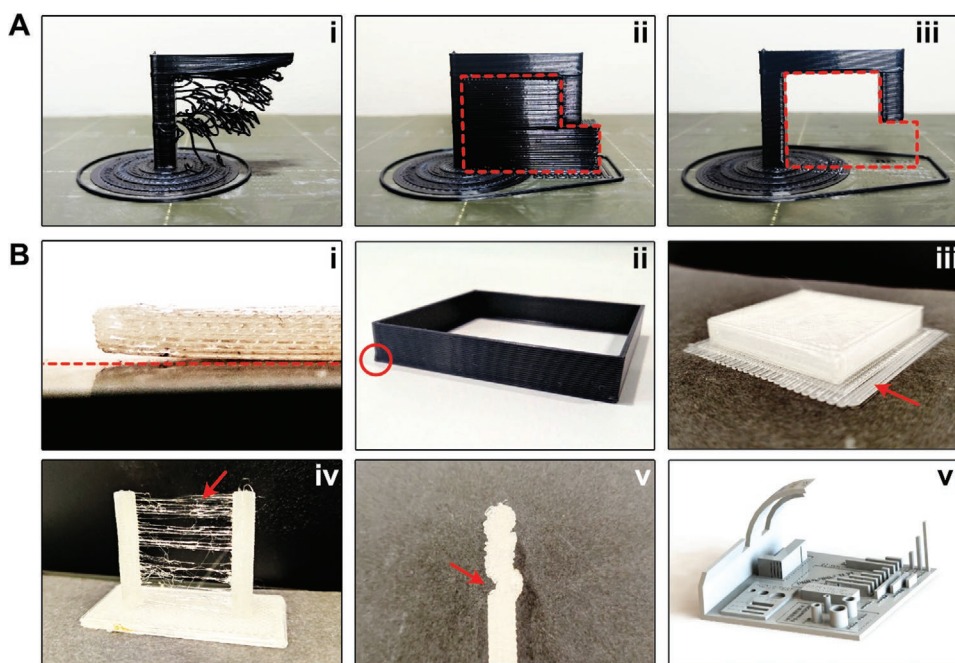
Figure 7. 3D printing limitations, defects, and artifacts. A) Models containing overhangs require support structures to be printed. i) Printing overhangs without support structures can result in defects. ii) Simple support structures (red dashed lines) allows the correct printing of overhang structures. iii) After printing, the support structures are removed to reveal the final shape. B) Printing defects and artifacts can result from a multitude of factors. i) "Warping" or "curling" is a deformation resulting from the materials' expansion coefficient. The red dashed line highlights the degree of deformation versus the printing plane. ii) High printing bed temperatures can lead to "Elephant's foot", consisting of a first layer wider than the subsequent layers. iii) Printing a raft can help avoid "warping" and "elephant's foot". (iv) "Stringing" or "oozing" results from material extrusion in between movements of the printing head. v) "Layer shifting" typically occurs when the printing head collides with the object during printing. vi) Several printing settings can be tuned to achieve a perfect printing job. The tuning process can be simplified by using open-source, free models available in online repositories (https://bit.ly/3tx6Oag).

flexibility, are usually inferior compared to FDM. To overcome this limitation, material manufacturers are constantly creating new formulations and, in some cases, were able to develop resins presenting comparable or even better characteristics than FDM materials in terms of flexibility or hardness, heat resistance, and solvent resistance. Due to its unique printing method, SLA can confer anisotropic properties to the printed materials and provide the highest possible resolution, accuracy, and smoothest surface of all 3D printing technologies. For this reason, SLA is widely used in the industry to create prototypes with intricate patterns, casting, and molding. SLA printers usually use a laser for curing the resin. This process is, however, slower and more complex than FDM printing. In addition to this, the layers of the SLA print can go as low as 25 μm, making the printing process slower than a standard FDM. In recent years, masked SLA (mSLA) printers have reached the consumer market. The chemical process is similar to the SLA-crosslinking resin using UV light; however, the way the light is processed differs. Whereas in the SLA, a laser is employed, in mSLA, a set of UV LEDs is used for the crosslinking process, and an LCD on top of them will control where the UV light passes, effectively using the transparent or black LCD pixels as mask lithography. Thanks to this ingenious method, those printers are equally or even more inexpensive than) FDM printers, and, as they cure one complete layer at a time without the need of moving a laser (or a printhead), they are also faster than SLA or FDM. The

inexpensive availability of those printers, together with their speed and precision, may open the possibility of using mSLA printers in the lab.[82] Clear/transparent materials are achievable with liquid resins. Both FDM and (m)SLA technologies are usually compared based on availability, cost, the durability of the materials, and the level of structural details provided (see **Table 1**).

Table 1. Commonly used 3D printing technologies for microscopy applications. Fused deposition modelling (FDM) and stereolithography (SLA) are two 3D printing technologies with different applications. FDM is generally more suitable for inexpensive, rapid prototypes with modest structural complexity and substantial mechanical properties. At the same time, SLA is more ideal for highly intricate objects where high mechanical impact is not present.

	FDM fused deposition modelling	(m)SLA stereolithography
Technology	Filament deposition by thermoplastic extrusion	Light curing of liquid resins
Materials	PLA, ABS, PETG	Resins
Costs	Relatively low	Higher than FDM
Pros	Affordable materials, printing of multiple colors and materials	Finer structural detail and complexity, quiet operation
Cons	Support structures required for complex geometries	Limited choice of materials, messy, requires postprocessing
Applications	Rapid prototyping, optomechanic parts	Cast for PDMS chambers, high-detail structures

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7.3. Materials for 3D Printing

Besides the printing technology and the 3D design, one crucial factor defining the quality and physical properties of the 3D-printed component is the material choice. Therefore, a lot of effort is taken to optimize material composition to increase printability and print quality, but also to enhance physical properties like strength, flexibility, and biocompatibility,[98] or optical properties.[99] FDM materials, polymer filaments are available commercially and require a balance of processing temperature, build speed, polymer melt rheology, and CAD shape parameters. FDM has been used to print polymers, polymer matrix composites (PMC), biocomposites, polymer ceramic composites (PCC), and fiber reinforced composites (FRC).[100] Given its broad appeal, FDM uses a large selection of thermoplastics commercially.

Common materials include standard plastics used for noncritical functions and engineering plastics that are suitable for high mechanical stress work due to their mechanical properties. The best starting materials for 3D printing are PLA and PETG for their simplicity of use and relatively low printing requirements. Most of these materials are well suited for general use due to their low melting temperature and cost. More advanced thermoplastics, known as high-performance polymers due to their improved chemical, thermal, and mechanical properties, are also available at much higher prices and should be used in critical applications. These polymers include polyether ether ketone (PEEK), polyetherketoneketone (PEKK), and polyetherimide (ULTEM).

A detailed description of the properties and applications of the filament materials used in FSM is provided in the next section.

7.3.1. FDM Material Guide

The thermoplastic ABS convinces by its low cost, easy processing, and high mechanical and chemical stability and is widely used for rapid prototyping.[150] Parameters such as infill density, layer thickness, orientation, raster angle, and air gaps are essential to strengthen the printed part.[151]

PLA is a thermoplastic with a lower impact strength than ABS but better overall tensile strength. The tensile strength is dictated mainly by the raster angle, width, and layer height. Reducing the layer height is necessary to avoid the formation of voids and improve the mechanical integrity of the structure.[152,153]

Nylon PA presents high chemical resistance, tensile strength, and flexibility. These mechanical properties even improve at higher temperatures.[154,155]

PET has good chemical and impact resistance. This clear thermoplastic is commonly used in disposable plastic bottles and packaging since it is non-toxic and biocompatible.[156] PET has the advantage of being a highly recyclable material. However, it is brittle, making PETG the candidate of choice when PET plastics are needed.[109,156]

PETG is a copolymer formed by polyethylene terephthalate (PET) and ethylene glycol. It is a high-impact and chemical-resistant thermoplastic that can be recycled.[157] It is also transparent and biocompatible. However, its low resistance against ultraviolet (UV) light results in discoloration and brittleness when exposed to it for long periods.[158]

PC is a thermoplastic that presents high durability, impact, and heat resistance. The material is also moderately flexible and transparent but difficult to print as it requires high temperatures to be appropriately extruded, resulting in cooling difficulties and proneness to warping.[159]

PEEK, PEI, and **PPSU** are high-performance polymers providing unrivalled mechanical, chemical, and thermal properties. It is commonly used in industry for highly wearable parts such as those in aircraft, cars, drones, and rockets[160] or biomedical applications.[86,87] Due to higher printing temperatures, specialized printers are required.[155]

TPE is a flexible rubber-like combination of elastomers and polymers that are also recyclable. It is highly flexible and soft, with excellent impact resistance and shock absorption.[161]

Composite materials contain supplements such as metals (e.g., copper and iron) or carbon-based elements (graphene or short carbon fibers). Supplementing with iron improves thermal conductivity, storage modulus, and glass transition temperature.[162] Short continuous composites show increased rigidity and strength, corrosion resistance, and improved chemical resistance.[163,164] Among these elements, carbon, Kevlar, and glass are also used alongside naturally found components such as basalt, jute, and bamboo.[165,166]

7.3.2. SLA Materials

Photocuring 3D printing methods use a small dose of energy in the form of light to trigger covalent crosslinking of the material. For the covalent crosslinking to occur, three key components are needed: the initial energy (light), the printing platform, and the photocurable resin serving as the base material. Decreasing the materials' energy requirement or increasing the printer's energy output results in faster printing, with the former option being the main focus of optimization.[14] Since light can only print a layer of limited thickness, multiple light exposures are needed.[101]

From the chemical perspective, the process of gelation results from the crosslinking of the photoresin's monomers. In this process, a light source functions as an energy initiator that triggers a photoinitiator (PI), resulting in polymerization. These PIs are usually single molecules that cleave radical fragments when exposed to the light of a specific wavelength.[101] Common PIs include phenylphosphine oxide (e.g., Irgacure)[14] and Acyl phosphine oxides (e.g., TPO and BAPO).[98] Other methods use two elements: a light-absorbing molecule and a co-initiator.[98]

Furthermore, PIs, such as ethyl 4-dimethylaminobenzoate (DMAB) and zinc tetraphenylporphyrin (ZnTPP), use visible light to achieve the same effect but display high toxicity.[102,103] However, other PIs have been used with better results in biocompatibility, such as 3-hydroxyflavone (3HF) 2018, which displays overall lower toxicity allowing biomedical applications. Another important aspect of SLA is the monomers used to form the 3D object. Originally, they were composed of combinations of diacrylates dissolved in liquid acrylate or methacrylate.[14] Many monomers are used in commercial and research applications; a few reviews can also be consulted for more in-depth information.[14,104,105]

Commercially speaking, many different light-curing resins are available at different prices and properties. However, many of these resins are proprietary, making it difficult to understand

Table 2. 3D printing materials and limitations. 3D printing has multiple advantages and disadvantages depending on the technology and materials used. Most importantly, these limitations can be circumvented by a proper understanding of the limitations of the materials.

	Material	Speed	Low cost	Heat resistance	Chemical resistance	High strength	Flexibility	High detail	Transparency	Bio-compatibility
FDM	ABS	◉	◉							a)
	PLA	◉	◉					◉		
	PETG	◉	◉							
	PEI, PPSU, and PEEK			◉	◉					
	Nylon PA				◉	◉	◉			
	TPA and TPU						◉			
(m)SLA	Standard resin		◉					◉		
	Clear resin							◉	◉	
	Castable resin							◉		
	Rubber-like resin					◉	◉	◉		
	Though resin					◉		◉		
	Bio-based resin			◉		◉		◉		
	Thermo resistant resin			◉				◉		
	Dental resin							◉		◉

a)Can be made biocompatible with post-processing.

the material's nature. Many commercial resins that mimic other plastic materials are sold as-is, often using their characteristics and similarities with existing polymers instead of disclosing their chemical contents. For this reason, in this review, resins are classified by their function and how they can be found commercially, without mentioning specific brands. Standard resins are the cheapest available and are very suitable for visual applications because they provide a smooth surface finish and a high printing detail level. Their main drawback is brittleness, which makes them incompatible with parts that suffer mechanical stress. Clear resins are helpful when transparent materials are needed but require additional post-processing to achieve a clear glass-like finish while suffering from brittleness.

Castable resins help create mold patterns and provide very high printing detail. Tough resins mimic ABS or PLA's mechanical and chemical properties; they are often slightly worse than their thermoplastic counterparts and don't provide the same thermoresistance properties. Temperature-resistant resins, on the other hand, are suitable for thermal applications and molding but generally expensive. Dental resins are usually used in biomedical settings and provide the best biocompatibility while maintaining good mechanical properties and high abrasion resistance. However, they are costly, and for microscopy applications, post-processing to increase biocompatibility is a viable option, as mentioned in the next section. The properties and suggested applications of these materials are summarized in **Table 2**.

8. Current 3D Printing Challenges and Limitations

3D printing presents great advantages in customization, design freedom, costs, accessibility, and the capacity to produce highly complex structures compared to other (micro)fabrication methods. However, these advantages are accompanied by drawbacks and challenges, including limited high-throughput application, low mechanical and anisotropic properties, printing errors, and design and material choice limitations. Additionally, it requires knowledge of 3D modelling and understanding the materials and the resolution needed. For projects involving direct contact with living organisms, it is vital to consider the material biocompatibility.[66] Beyond the biocompatibility aspect, there is an increased concern about the potential environmental impact caused by 3D printing, as the process consumes large amounts of energy, produces plastic waste, and generates air pollution.

8.1. 3D Design and Fabrication

The successful design and execution of a 3D printing project can be challenging, as various unexpected and unwanted defects in the object shape can emerge as a result of the fabrication process. 3D printed parts often carry artifacts depending on the object's geometry, such as in the case of an insufficient polygon-approximation of curved surfaces.[95] Post-processing can often alleviate these defects, but good design practices are required to minimize them. As discussed in the section on 3D design, it is essential to optimize the printing orientation, create supporting structures when the design requires it, and add enough layers in the slicing step.

The "layer-on-layer" nature of FDM printing can be a limiting factor in specific designs. For example, some designs contain features called "bridges", requiring horizontal material deposition between two raised points (**Figure 8**A). Printing perfectly flat bridges is often needed. However, since FDM requires the printing material to be melted at high temperatures and then hardened by cooling, the bridge layers tend to become deformed due to gravity between these two stages. Thus, "bridging" can be troublesome and sometimes even impossible to achieve. Naturally, common strategies to improve "bridging" include increasing cooling, decreasing material extrusion rate, decreasing nozzle temperature, and decreasing printing speed.
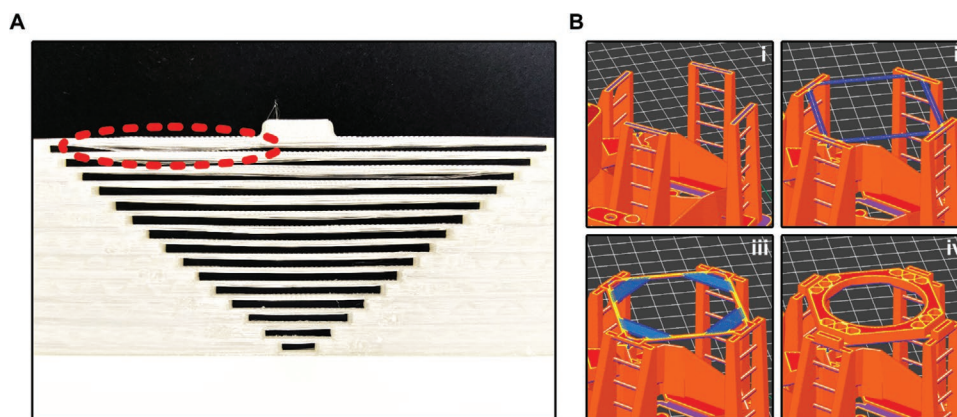
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Figure 8. 3D printed bridges in FDM. A) Printing bridges requires horizontal material deposition between two raised points. "Bridging" can be troublesome because the material needs to be melted at high temperatures to be extruded and then cooled down to harden. Thus, bridges tend to be deformed due to gravity when cooling is insufficient or the length of the bridge is large (red dashed box). B) i) The OpenFlexure microscope base features an elevated platform between four columns that would typically require support structures to be printed. The design employs a "bridging" strategy to avoid the use of support structures. ii) A "bridging" layer is first printed to connect the closest points between the columns. iii) A second layer of bridges is then printed in a different orientation. iv) The last layer serves as a base on which the elevated platform is printed.

Furthermore, adding support structures to the design is a common approach to circumvent this limitation. However, these structures need to be removed after printing is finished, resulting in increased post-processing times. Also, certain features are too structurally complex to include support structures or to allow their removal without resulting in substantial harmful effects. An interesting example of how the inclusion of support structures can be avoided by design is the OpenFlexure microscope base. In this model, an elevated platform needs to be printed between four columns without directly contacting them. The model becomes printable without support structures by including a bottom layer that bridges the closest points between the columns, upon which another bridging layer is printed in a different orientation. This last layer serves as the floor on which the elevated platform is printed (Figure 8B-i–iv).

An essential challenge during printing is the formation of voids between material layers. During printing, layer deposition can create unwanted porosity. This porosity often reduces the printed object's mechanical properties[106] and this is influenced by choice of material and technology used, with FDM having void issues more commonly than SLA.[106] In FDM, reducing porosity during printing requires an increase in the printed object wall thickness. However, this approach also reduces the final product's tensile strength alongside further design issues.[107] Interestingly, this 3D printing flaw can also be exploited by controlling the porosity to develop porous scaffolds employed in tissue engineering applications.[108]

The mechanical properties and anisotropy of structures also present a challenge, as each printed layer is not the same as the one before. This property often results in unwanted mechanical behavior, particularly when vertical tension or compression is exerted on the printed object. This is more common in thermoplastics printed with FDM.[109] Another factor affecting tensile strength is the printing orientation. For example, the printing angle presents a relationship with the elasticity of the final product when using ABS.[110] FDM also results in the appearance of layers in the final printed product. This is perhaps not an essential factor when the part is not visible, but the exterior

details often need post-processing, such as sintering, to correct this problem.[95] For SLA, this is usually not an issue.

The multitude of design and fabrication aspects mentioned in this section highlight that design is crucial for 3D printing. Concerns on the technology's implementation and projects' feasibility often revolve around practical and monetary aspects. However, the human skills required to use 3D printing as a tool should also be addressed in "cost". In particular, 3D design skills are necessary to build new models or make alterations to previous models, the latter being crucial for prototyping. Since these are relatively complex skills that fall outside most researchers' scope, acquiring them might require additional effort. While it is possible to undertake paid courses on 3D design, the substantial amount of literature available online enables newcomers to learn independently (e.g., https://wiki-factory.com/+wikifactory/stories/ultimate-guide-how-to-design-for-3d-printing). Nonetheless, depending on the complexity of each user's goals, the time taken to learn the required skills needs to be accounted for as a possible challenge. For most applications, this task is achieved relatively quickly but often involves a process of trial-and-error that can slightly increase overall costs (e.g., extra filament and energy).

8.2. Biocompatibility

Regardless of the material and 3D printing technology used, if the 3D printed object will come into contact with biological samples and tissues, particularly in a medical setting, the components must abide by the ISO 10 993. This certification comprises several standards for evaluating medical devices and material biocompatibility to assess and manage biological risk.[111] Polymers can be toxic when in contact with biological tissues due to mechanical or chemical degradation. In this review, material toxicity in organisms, tissues, and cells during imaging approaches are discussed. Reviews that deal with long-term exposure to 3D printed materials can also be found in biomedical research reviews.[112–115]

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Given that FDM is the most user-friendly 3D printing technology, it is reasonable to adopt it for biological purposes. Previous work has employed materials such as ABS,[116] PC,[117] and PET[118] with little to no toxicity when used along with collagen coatings as a substrate (ABS and PC) or just by washing (PET). Another method tested was UV light treatment, where ABS-like materials were successfully made compatible with zebrafish embryos.[119]

On the other hand, photopolymers are often more toxic due to their nature and the potential residues that remain following the photo-curing processes.[66] Several studies considered 3D printed objects using SLA technology as toxic when used directly in biological applications without post-processing.[120–122] Accordingly, photoinitiators (e.g., BAPO and TPO) are known to have cytotoxic effects at low micromolar concentrations in human cells, leading to mutations and genetic instability.[123] Similar to FDM-based printed objects, improving biocompatibility requires postprocessing. There are a variety of post-processing methods available which can be adapted depending on the nature of the resin material or its function. One of the reasons suggested to underlie toxicity is the chemical composition of the 3D printing material itself. Often, the complete formulation of these materials is only known to the manufacturers, but photoinitiators and acrylate monomers are toxic for living organisms.[121] Material Residue remaining on the printed objects' surface can be washed with ethanol, sonication, and sterilized with UV light.[121] Another study identified uncured residual monomers in the objects' surface using HPLC-MS, explaining their high toxicity and suggests combining residual photopolymer extraction via supercritical CO2 treatment and post-curing.[120] UV light has also been successfully used to reduce toxicity, perhaps by finishing curing any uncured residues.[119] Using a nitrogen atmosphere and high temperature is also a practical approach to detoxify material. However, it negatively impacts the material's transparency, making it incompatible with clear/transparent resins.[122]

Lately, surface coating with hydrophobic epoxy resin has been shown to improve biocompatibility.[85,124] Although epoxy resins require extra steps of preparation, they can dramatically increase the printed object's biocompatibility up to the standard of commercially available cell culture vessels. These steps include curing the resin by heating overnight at 45 °C followed by PBS and ethanol washing steps. Epoxy resins are also compatible with transparent materials, making them a suitable candidate to combine with cell culture vessels.[85]

8.3. Autofluorescence

An important aspect to consider in materials used for microscopy applications is autofluorescence. Autofluorescence is the tendency of a material to emit fluorescence when illuminated at specific wavelengths. This property is problematic in imaging applications because many materials are autofluorescent, and the autofluorescence spectra often overlap with the common spectra chosen to image samples.[125] Testing the materials for autofluorescence is a general recommendation for 3D printing materials used in microscopy applications to minimize this effect.

8.4. Environmental Impact

The environmental impact of 3D printing processes is still an ongoing topic of discussion within the field.[126] To address the environmental impact of 3D printing, three aspects will be discussed in this review: energy consumption, waste management, and air pollution.[6] Energy consumption is considered the factor with the most significant impact, particularly in mass production.[16] Current 3D printers are not highly optimized for energy consumption[127] and depend on material choice, build volume, layer thickness, and printing speed. This optimization lies partially in the often-overlooked cooling systems of 3D printers, which mostly rely on electrically powered fans to dissipate heat.[128]

Material waste is also a significant factor in the environmental impact of 3D printing. The two biggest challenges in this regard are the use of recycled materials and recycling waste products. Using recycled material for 3D printing is not impossible, as several materials, such as PLA and ABS, can be processed and turned into filaments again.[129] Recycled PLA or PETG have also started appearing on the market. From the consumer point, the use of support material should be considered. As this material is thrashed right after the print, it would be environmentally beneficial to design a 3D printed part that would use as little support as possible or none at all. Regarding the recycling of material, it has been suggested that a resin identification code should be added to recycling guidelines, particularly as the technology becomes more common in domestic settings.[6] For FDM, the material can be processed into small pieces and then reformed into filaments using a heated extruder. The downside is that the heating process destroys the chemical bonds of the polymer, making it weaker. To improve the strength of recycled materials, small amounts of non-recycled material can be added.[7]

Finally, indoor air pollution is also a concern in 3D printing. The thermoplastic extrusion process can generate particles of volatile organic compounds (VOC), which can be dangerous to humans and the environment.[129] This is particularly hazardous in enclosed environments, and protective clothing and masks are recommended, as volatile organic compounds (VOC) emissions are related to the thermal degradation of the polymers and additives used in the 3D printing material. One potential solution is using different low-toxicity thermoplastics such as bio-based ones (PLA) and avoiding fossil fuels produced plastics (ABS) since they require lower temperatures to melt printing, reducing emissions and energy consumption.

In contrast, 3D printing can positively impact the environment, as this technology allows more efficient fabrication than traditional manufacturing methods, providing a way to optimize industrial processes. The design of the 3D printed part is also essential, as parameters such as layer thickness or printing orientation can demand more energy, requiring fine-tuning to optimize energy consumption as much as possible. Another option is to implement greener alternatives as 3D printing materials, reducing the printing process's environmental impact.[130] 3D printing is still a young technology, and there is ample room for optimization, particularly in energy consumption and cooling systems, recycling of materials, and emissions.

As technology becomes more standardized, machine design and printing processes are expected to improve.

9. Outlook

Additive manufacturing is revolutionizing industry and research, and it will possibly become the technology of choice for manufacturing processes. This review aims to introduce researchers to the advantages and limitations of 3D printing by giving a comprehensive view of the application of commercial printers in optical microscopy for life science. The versatility of additive manufacturing technologies and the relative accessibility will only increase with time, as the field is still in its infancy.

The obstacles that 3D printing currently faces involve the 3D design of objects, available materials, biocompatibility, energy consumption, waste management, pollution, and large-scale applications. However, these minor setbacks are expected to be improved as the technology becomes more common and easier to use. Another limitation lies in the materials used for 3D printing. Fortunately, materials with high mechanical and chemical resistance that are also less toxic are actively researched in industrial and academic settings. Biomedical research is, for example, providing great strides in terms of biocompatible materials and coatings, particularly as 3D printing is a prime candidate for implant manufacturing.

Environmental impact is a key factor for the use of mass production in the industry. Energy consumption will likely reduce with optimization of the printing processes, as current 3D printers are not heavily optimized in terms of energy consumption. Plastic waste management is also standardizing recycling processes to take full advantage of discarded printing materials. Pollution also depends on the optimization of the printing processes and will likely see a reduction with time. Large-scale applications will follow once 3D printing processes become more efficient and cheaper, which will facilitate the accessibility of the technology. Despite current limitations, 3D printing is already enabling unprecedented customization levels, allowing researchers to design and produce tools tailored for specific needs for low prices. 3D printing in microscopy development is particularly exploiting these capabilities, as it enables rapid prototyping and design iteration that would be otherwise difficult -if not impossible- with other fabrication technologies.

9.1. The Future and Reach of 3D Printing

The examples of 3D printing's applications provided in this review highlight how its emergence revolutionized the manufacturing landscape. Beyond the applications in optical microscopy, there are 3D printing applications in bioprinting,[131–134] lab-on-a-chip,[135] and organ-on-a-chip.[136–149] Most 3D printing techniques rely on affordable equipment and materials, bringing the accessibility of automated manufacturing processes previously exclusive to big companies down to the "hobbyist" level. Furthermore, the relatively easy access to scientific devices such as 3D-printed microscopes promotes "Citizen Science" by providing data collection tools to users who would otherwise be unable to purchase them. Thus, the manufacturing process becomes possible and cost-efficient for small- to large-scale private projects. This autonomy precludes the need for outsourcing and the dependency on proprietary formulations or devices, which are often detrimental to project budgets and substantial limiting factors. Notably, the affordability of 3D printing technologies enables low-income countries to benefit from its advantages and the devices that it can produce. The freedom to design 3D models using open-source CAD and slicing software allows customization, facilitates prototyping, and enables sharing of designs between researchers. Furthermore, the different printing technologies and materials available, coupled with the control over several printing settings/parameters, allow users to manage crucial factors influencing their models' applicability, such as structural integrity and detail.

The establishment of 3D printing in a multitude of novel applications results in an unprecedented exploration of the technology's potential and limitations. In turn, this generates a driving force for its development based on necessity. Thus, the overall advantages and limitations of 3D printing are expected to improve substantially in the future. Also, the relevance of the current 3D printing applications and the need to overcome their limitations make 3D printing technology a high-impact research field. In this sense, microscopy is expected to continue to benefit from developments in 3D printing technologies. The development of new materials can improve current applications' success, for example, by providing biocompatible materials for sample manipulation. However, this requires the collaboration of material scientists and microscopy researchers. Furthermore, 3D printing is an ideal platform to produce compliant mechanisms, which can improve current mechanical designs underlying the function of specific microscope parts. In particular, the high degree of movement precision and mechanism miniaturization that can be achieved with 3D-printed compliant mechanisms make them a promising venue for developments in stage and sample micromovement applications.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

M.D.R., H.S.H., and A.M. contributed equally to this work. M.D., H.S.H., and A.M. contributed equally to preparing the manuscript, writing the document, preparing figures, tables, and movies. All authors planned structure, advised in the preparation, and edited content.

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