The maker movement has reached the optics labs, empowering researchers to actively create and modify microscope designs and imaging accessories. 3D printing has especially had a disruptive impact on the field, as it entails an accessible new approach in fabrication technologies, namely additive manufacturing, making prototyping in the lab available at low cost. Examples of this trend are taking advantage of the easy availability of 3D printing technology. For example, inexpensive microscopes for education have been designed, such as the FlyPi (1). Also, the highly complex robotic microscope OpenFlexure (2) represents a clear desire for the democratisation of this technology. 3D printing facilitates new and powerful approaches to science and promotes collaboration between researchers, as 3D designs are easily shared. This holds the unique possibility to extend the open-access concept from knowledge to technology, allowing researchers from everywhere to use and extend model structures. Here we present a review of additive manufacturing applications in microscopy, 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.

Introduction

From the first 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 (3). Recent years have seen a sharp increase in bespoke microscopes, achieving excellent results where commercial solutions were ineffective, for example the Warwick Open Source Microscope (WOSM) (https://wosmic.org) and the OMX Microscope (4). However, researchers are often limited to commercial parts, as fabrication methods to reliably manufacture microscope components are expensive and slow. 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 the field of manufacturing. This impact is based on its rapid prototyping approach to the creation of physical objects, called additive manufacturing. Additive manufacturing has seen a massive rise in popularity in recent years, from its start as an industrial prototyping tool to the current domestic use. In 3D printing, a structure is built bottom-up in an...
additive manner by depositing sub-mm layers of material. In contrast, conventional manufacturing processes usually rely on manual labour and automated processes such as casting, forming, and machining by either subtracting material from a larger starting piece or using moulds to shape an object (7). The additive manufacturing approach pursued in 3D printing uses simplified one-step manufacturing processes, reducing material waste, which in subtractive fabrication methods such as milling can be 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. These components can be assembled to create complex machinery such as microscopes (2). 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, as 3D designs are easily shared. 3D printing holds the unique possibility to extend the open-access concept from knowledge to technology, allowing researchers and people with a keen interest in science to use, iterate designs and adapt existing ones for different projects with a high degree of customisation.

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. Fig. 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 strong objects (as done in FDM), or using light to selectively polymerise resins step by step to obtain highly intricate and complex forms (as done in SLA) or masked SLA (mSLA) (9).

Additive manufacturing starts with a 3D model designed from scratch or downloaded from the internet (Fig. 1). This is an exciting aspect of the technology, as files can be easily shared online, greatly increasing accessibility. The files are created and visualised in commercial or open-access 3D software (see also "Box 1. Software tools"). Once the 3D model is ready, it needs to be converted into a “.STL” file, which stands for Standard Tessellation Language. This is usually 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 in 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 1. Software tools" below).

Once a g-code file is created, the next step is 3D printing. The selection of materials and 3D printing technology will 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 (10). Depending on the machine used and the printing job, the printing process can be lengthy, taking over 1 day in some instances to complete. Once the object is printed, it can be subjected to post-processing steps.

Box 1. 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 inexperienced users, browser based (www.tinkercad.com)

OpenSCAD: Free, creation and rendering, modeler based (www.freescadweb.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, handle 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)
cent years, 3D printing-based solutions drastically increased and microscopy to create open access and inexpensive technologies are creating an impact in two critical aspects: accessibility and rapid prototyping of customised equipment. Along with the exception of a few parts, it is currently possible to print whole microscope bodies at relatively low prices. Additionally, 3D printing is used for sample control such as microfluidics systems and sample holders.

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 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.

### 3D printing in microscopy

With regard to the field of microscopy, 3D printing technologies are creating an impact in two critical aspects: accessibility and rapid prototyping of customised 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, down to the level of molecular organisation. From the very early days where Antonie van Leeuwenhoek observed protists and bacteria for the first time (11), to the modern super-resolution solutions capable of resolving images below the light diffraction limit (~ 300 nm), microscopy is the backbone of biology research (12).

Microscopy technology today is currently still limited in its application, often requiring high investment and specialised training. This puts researchers with limited funds at a disadvantage, granting access to sophisticated imaging approaches only to researchers from wealthy countries and facilities. This has given rise to a new movement in microfabrication and microscopy to create open access and inexpensive technology that can be accessible to a wider 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 wider 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 well, 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 crowd sourcing, which benefits society.

Many examples of this trend are taking advantage of the easy accessibility of 3D printing technology (see also Movie S1). For example, inexpensive microscopes for education are being produced, such as the FlyPi (1). Also, the highly complex robotic microscope OpenFlexure (3) represents a clear desire to democratise 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 the generation of 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 (Fig. 2).

These characteristics enable systems such as miCube (13), the UC2 (14), and the the Warwick Open Source Microscope (WOSM) (https://wosmic.org), where further customisation 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 (13, 15), the body (13, 15, 16), holders for the filters (15, 17), objectives (18), coverslips (14), pinholes (14, 16) and heat sinks (18). Additionally, microscope chambers (19) and controller mounts (20) have also been implemented, allowing a high degree of customisation to researchers that adopt 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 that includes sample holders, sample surveying, and microfluidics systems (see also Movie S2). 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 (21) and incubation chambers (22). Sample surveying using Atomic Force Microscopy cantilevers has also been 3D printed (23). 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 of automatic miniature pumps and Lab-on-Chip devices. Additionally, pump systems have been fully automated to allow sample manipula-
tion for downstream microscopy applications such as fixation and immunofluorescence (24, 25).

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. The implementation of 3D printing approaches in the rapid fabrication and prototyping of optomechanical components allows the creation of 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. Using additive manufacturing to produce specific microscope components is highly beneficial, allowing fine-tuning to suit specific design requirements. It has been reported that 3D printing can reduce a microscope parts’ price between 50% and 90%, depending on the component (26). The performance of the 3D printed parts (kinematic mounts, translation stages, and integrating spheres) were directly compared to commercial counterparts to assess their precision performance. One of the tradeoffs that has to be accepted is the printed part’s limited physical integrity, which can be compared to low-end commercial alternatives (26). 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 sub-micron-scale motion with remarkably low drift and minimal post-processing (27). Micromanipulators and probe position systems have also been 3D printed and tested using flexible materials with substantial price reductions when compared to commercial options (28). Even tunable objectives have even been realized by aligning customised poly (methyl methacrylate) (PMMA) singlets and a miniaturized electrowetted lens for electronic focusing within a 3D-printed housing (29). 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 to realize a phasemask based on a 3D-printed mould with micrometer topography (30). Beyond this, low cost, compact and high-performance illumination systems have been realized employing 3D printed components, such as the NicoLase project. 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 (20). The increase in mobile phone usage backed by powerful cameras and increasing computational output has given rise to the field of 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 both acquisition and processing is now possible in the same device (31).

This practice can be particularly useful 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. 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 artefacts (31). As the high performance scientific camera is usually one of the most expensive components of a microscope, exchanging it for a mobile phone camera allows to significantly reduce the overall costs of the system, even more so by integrating this approach into a 3D-printed microscope. This approach has been pursued for the FPscope project, capable of high-resolution imaging using variably illuminated, low-resolution intensity images in Fourier space, called Fourier ptychographic microscopy. Here, the mobile phone lens is used in a reversed manner where the mobile phone lens projects the magnified image to the detector (32). The pSmartScope is another example of a 3D printing adaptor that can be fitted into a wide range of mobile smartphones. In addition, the motorised stage is fully automated and controllable by the smartphone and is capable of autonomous image acquisition (33).

Especially for particular tasks where the limited camera performance and computation power is sufficient, smartphones are used for microscopy applications in enabling point-of-care and field diagnostics. These kinds of applications are for example DNA fluorescence spectroscopy for the read-out of fluorescence-based biological assays to detect specific nucleic acid sequences reached the point of detecting single-base mutations (34). 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. Following product amplification, end-point detection is achieved using the smartphone-based fluorescence microscope (35). Automated cell identification has also been done using a 3D printed shearing digital holographic microscope for fieldwork. 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 (36). Another holographic lens-less smartphone-based microscope has been designed for holographic micro-object imaging of a variety of samples using a CMOS camera chip and controlled by a Raspberry Pi (16). Further examples of smartphone-based projects differentiate between white blood cells with acridine orange staining using a miniature achromatic microscope (37), identify pathogenic bacteria using a DNA-based FISH assay (38), 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 (39).

Perhaps the most interesting application of 3D printing in microscopy is the generation of fully functioning microscopes with few non-3D printed components. 3D printed microscopes pose an exciting concept that combines iterative and
collaborative design, resulting in highly customisable equipment that would be very expensive to obtain using other fabrication methods. Thus, beyond the iterative design aspect, many 3D printed microscopes are open-source projects that present a unique opportunity to provide accessibility to novel ground-breaking designs to a wide range of researchers bypassing geographical and economic limitations.

Fully functioning 3D printed microscopes often present a modular design, allowing 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. Additionally, it facilitates the alteration of the original design, allowing to generate new concepts (41).

One of the most prominent examples of open-source microscopy projects is the OpenFlexure microscope (Fig. 3a). OpenFlexure is a fully automated laboratory-grade microscope capable of using motorised sample positioning and focus control (3). Additionally, it is highly customisable, 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) that was calibrated to use custom optics (42). 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 that interface with each other using a web API with the 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 (43). 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 (2). The OpenFlexure project has also been combined with SRRF (44) for super-resolution applications (45).

Another example of a 3D printed microscope with super-resolution capacity is the Chea(i)p, a self-contained super-resolution microscope that uses a commercial objective and a mobile phone, and costs less than €800. In this case, the mobile phone does the acquisition, processing, hardware control, and photonic-chip illumination. Impressively, it can reach resolutions of 100 nm with SMLM and live super-resolution with SRRF (46). Furthermore, the waveguide-PAINT system allows a highly uniform 100 x 2000 µm² 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 is capable of imaging multiple whole cells or whole origami structures such as microtubules in COS-7 cells in a single field of view (47).

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 (18). Beyond applications in education and scientific outreach (48), the system can be upgraded and adapted for specific requirements and budgets. Although considered a challenging endeavour, the OpenSPIM was adapted and used to image the organism Maritigrella crozieri using two-colour laser illumination to simultaneously detect two probes at the same time (49). Another SPIM 3D printed system is the Flamingo, which offers 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 (2). The OpenFlexure project has also been combined with SRRF (44) for super-resolution applications (45).
tion, allowing to bypass the necessity of precise mechanical focusing during acquisition (50).

One of the goals to achieve wide accessibility to microscopy technology is to substantially reduce the price by either 3D printing or procuring commonly used laboratory parts. Here great examples are the OPN Scope that aims to lower the costs for fluorescence microscopy (51) 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. The system was tested in experiments involving behavioural tracking in C. elegans as well as Optogenetics and Thermogenetics in Drosophila and C. elegans (1). The miCube open-microscopy framework is another system of this family. It has been used to visualise dCas9 in vivo target search, as it is capable of single-molecule microscopy with high spatiotemporal resolution (13). The Microscopi project aims to democratise microscopy with their portable, low cost, 3D printed and self-built systems capable of multimodal imaging (bright field, dark field, pseudo-phase and fluorescent microscopy). It uses an automated XYZt imaging system controlled by a tablet or smartphone using a simple GUI (52).

Lastly, the UC2 (You. See. Too.) project is a low-cost, highly versatile and customisable 3D printed microscope that uses a modular design toolbox. The system is fully accessible online and uses many common off-the-shelf optics and electronic components that are fitted in 3D printed cubes (Fig. 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 (14).

Sample Manipulation, microscopy and 3D printing

3D printing applications for sample manipulation are a highly versatile and powerful tool. Especially the field of microfluidics is a highly interesting target for applying additive manufacturing, allowing to downscale biochemical applications to the point of creating portable and nano-scale versions of a testing laboratory. While originally the field was based on moulding and replica molding fabrication, today 3D printing allows the creation of more complex geometries and designs. With this approach, the production of moving parts is possible, including miniaturised pumps and valves that allow accurate fluids control, as well as sensors that allow the detection of microchanges in the environment (Fig. 4). Additionally, the variety of 3D printing materials available allows researchers to customise the parts to suit specific needs (53). Functional parts 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 flow when the inside and outside channels are aligned in the opened position (53, 54). Other more complex valves such as pneumatic ones, can be printed in arrays to mimic circuits (55) or quake valves (56). Another method to control microfluidics is using pumps. Syringe pumps are simple and can be used to deliver precise volumes of liquids for various research needs (e.g., delivery of drugs to samples during live imaging).

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 (Fig. 4a) (24, 25). The highly modular lego-and-3D-printed system NanoJ-Fluidics (aka “Pumpy”) is capable of managing up to 128 syringe pumps simultaneously (24). These systems are controlled by Arduino controllers, allowing precise fluid volume manipulation.

Similarly to syringe pumps, fully 3D printed pumps are capable of inducing flow (58). 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 effective and can be printed using common FDM or SLA 3D printers (Fig. 4b) (59). These pumps can be employed in microfluidic automation and printing using designs that do not require posterior
assembly (i.e., “print-in-place”) (60). Furthermore, they can also be operated with Arduino controllers, allowing custom flow profiles for handling precise liquid volumes (57, 61).

3D printed pumps also are inexpensive to print, with prices going as low as €38 per channel (61). Peristaltic pumps 3D-printed using SLA can present durability issues. However, using thermal initiators in the liquid resin and post-processing baking of the component have been shown to greatly improve durability (62).

Some of these pneumatic pumps require complex ancillary control tubing. However, multiplexers allow 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 (58). Microfluidic chips can work as sensors, detecting physical and chemical changes inside chambers by measuring volumes on the picoliter scale (63–66).

Microfluidics devices are currently fully 3D printable (67) with the caveat of not being able to provide transparency (58). Printing glass-like transparent material is still not fully attainable and severely limits their microscopy applications. To circumvent this issue, certain parts, such as valves, can be 3D printed and then combined with glass or other transparent materials such as glass or polydimethylsiloxane (PDMS).

Here, for example, 3D-printed scaffolds made of ABS or water-soluble polyvinyl alcohol (PVA) filaments have been used. A mould is then used to cover the scaffolds with PDMS. As soon as the PDMS has hardened, water is used to dissolve the PVA filaments without leaving residues, while ABS can be dissolved by acetone. This approach presents the advantage that it allows to print directly onto the coverslips while also providing transparency, allowing excellent applicability for imaging (68, 69). Besides lowering costs, one of the advantages of using 3D printing when compared to other microfabrication methods is the more complex channel designs that can be achieved, such as serpentine flow channels with cross-sectional areas (67) and the compatibility with additional microfabrication approaches like micropatterning (70). Additionally, by bonding printed channels to transparent PMMA sheets, it is possible to produce highly complex arrays, such as straight, spiral, serpentine, curvilinear, and contraction-expansion (71). In some cases, designs such as T-shape pillars are not possible to obtain via other traditional fabrication methods in a single demolding step, but only by using commercial 3D printers (72).

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. A colourimetric assay based on the interaction between the MRSA mecA gene and AuNP probes was used to confirm the bacterium’s presence in the samples (73). An important limitation of the generation of effective microfluidic chambers on these chips is the dimension of the channels (71). The Micraft printer was designed with this problem in mind and is capable of printing complete microfluidic chambers for lower prices when compared to other methods. 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 (74). Microfluidic chips are perhaps the most promising in the field of “organ-on-chip” research, where whole organ-like functions are mimicked using a combination of cell types growing on chambers and channels inside the chip. For example, 3D tissue-engineered skeletal muscle (TESM) cultures have been established using commercial 3D printers by using PDMS to create chambers where cells can grow. Once the 3D cell cultures were established in the chamber, a suspension of hiPSC-derived myogenic progenitors and biocompatible hydrogels made of fibrin and Matrigel (75) was introduced. Differentiation resulted in the successful formation of a TESM culture with a comparable composition to other cultures, including myofibers (72). In addition, organ-on-chip managed to mimic liver interstitial structures containing endothelial cells and primary hepatocytes (76), produce a drug screening assay to assess cell reaction (77), and test for injury (78), hepatitis B infections and even alcohol-driven injury (79). Organoids for live imaging have also been developed in microfluidic chips combined with a bioreactor. The bioreactor provides ideal conditions to grow cerebral organoids to image the organoid self-assembly dynamics without transferring or disturbance. This approach facilitates examination of the sample while also allowing the delivery of drugs to modulate organoid behaviour (80). Other organs have also been modelled, such as the lungs (81, 82), kidneys (83, 84), intestine (85, 86) and heart (87, 88).

**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 together with epoxy resin appears to provide the best results, even when using clear materials (89). Depending on the sample holder’s nature or biological cage, autoclavable material might be useful for prior and post-sterilisation during experiments. Structures with these characteristics require printing with advanced plastics that provide high steam, temperature and physical resistance (90, 91). More information on material properties and their biocompatibility can be found in Table 2.

An advantage of using customised sample holders and biological cages is the possibility of tailoring them to fit different imaging platforms. Microscope parts are often expensive and incompatible between different manufacturers or machines. This limits for example cross-instrument compatibly 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 the growth...
of living tissue and easy adaptation between microscopy systems (92). This versatile sample chamber was 3D-printed using SLA and a biocompatible HTM140 resin from Envisiontech (Fig. 5a). The authors agreed that the system can be adapted to use any standard resins since glass and PDMS are the only materials in direct contact with the sample.

UniverSlide was devised to have the dimensions of a regular microscope glass slide (e.g., 26 x 76 mm²) 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 (Fig. 5a). The sample chamber can then be filled with cell culturing medium and used for microscopy applications in unicellular and multicellular samples. Also, it is compatible with live and fixed samples (92).

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. 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 (94). Direct sample manipulation is also possible, for example, using tension and other mechanical stimuli to determine tissue 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 (95) 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. Additionally, it is compatible with upright and inverted fluorescence microscopes and can perform up to 4.5 cm XY-stretches using an electronic controller (Fig. 5b) (93).

Another cell manipulation method is Atomic Force Microscopy (AFM), which uses a cantilever that provides data on cellular characteristics such as adhesion strength, elastic modulus, and mechanobiological properties (96–98). AFM can also be combined with microfluidics (99), allowing accurate force control and fluid manipulation inside cells. This type of setup uses an AFM cantilever with an aperture at the tip and an internal channel. This microfluidics cantilever was made using SLA and two-photon polymerization (2PP printing on a SL-printed fluidic interface). This type of cantilever probes provide microfluidics with AFM functionality and are useful for precise fluid manipulation inside cells via cell puncturing (100).

3D printing resources and technology in microscopy applications

This review aims to enable researchers who are unfamiliar with 3D printing to implement this approach for their own microscopy projects by providing a comprehensive guide of available resources and technologies, including a quick start guide (see "Box 3. How to 3D print" on page 16).

Databases for 3D parts. 3D printing databases exist for both research (e.g., NIH Exchange: a database of 3D printed parts) and more general applications (e.g., Thingiverse), providing 3D printing enthusiasts the means to obtain complete 3D models. However, a complete database focused on microscopy projects does not exist. This represents a substantial obstacle for newcomers, as information is not centralised and can be difficult 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.

Commonly used 3D printing technologies. Additive manufacturing comprises a variety of different technologies. Although the technology itself is widely known and highly advertised, researchers unfamiliar with 3D printing technology still struggle to fully realise its potential beyond a 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 (9). From these widely available printers, FDM and SLA stand out as the simplest and most inexpensive options for a beginner and thus, will be the focus of this review.
**Fused Deposition Modeling (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 materialise the design. The printer itself consists of a platform, 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 3-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. This is followed by the head or the print bed moving in the z-axis according to the layer thickness, and the addition of another layer using x-y axis movements as before. 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. A wide variety of materials is available, of which the most common are acrylonitrile-butadiene-styrene (ABS), polylactic acid (PLA), Polyethylene Terephthalate Glycol modified (PETG) polycarbonate (PC), polyamides (PA), and Polypropylene (PP). Additionally, FDM enables colour printing if filaments with different pigments are used.

Furthermore, other elements besides the materials used contribute to the integrity and properties of the prints (Fig. 6a). Several studies have experimented with path-planning and part-orientation to alter the anisotropic mechanical properties of the 3D printed parts (101). 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) (Fig. 6b). Importantly, 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 (102). However, while “rectilinear” excels at resisting forces applied in the direction of the pattern’s lines, it is extremely weak 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 (Fig. 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 degrees require the use of supports to be printed correctly (Fig. 7a). 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 smoothed (103).

Several printing artifacts can result from a multitude of factors (Fig. 7b). “Warping” is a curling deformation often accompanied by a partial or complete detachment of the object from the printing bed (Fig. 7bi). 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 material’s 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), and it can be minimised 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” (Fig 7bii). 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 the extrusion of material beyond the predicted line width. Thus, “elephant’s foot” is typically solved by adjusting the printer’s settings and environment. Another solution for warping and “elephant’s foot” is printing a raft, which is an additional and wider first layer meant to be damaged in place of the original first layer (Fig. 7biii). 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 material is extruded while the nozzle is moving to a new location (Fig. 7biv). Consequently, thin...
Table 1. Commonly used 3D printing technologies for microscopy applications. Fused deposition modeling (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, while SLA is more suitable for highly intricate objects where high mechanical impact is not present.

| Technology | FDM | (m)SLA |
|------------|-----|--------|
| Filament deposition by thermoplastic extrusion | Light curing of liquid resins | |
| Materials | PLA, ABS, PLA, PETG | Resins |
| Costs | Relatively low | SLA higher than FDM, (m)SLA comparable to FDM |
| Pros | Affordable materials, printing of multiple colours and materials | Finer structural detail and complexity, quiet operation |
| Cons | Support structures required for complex geometries | Limited choice of materials, untidy, requires postprocessing |
| Applications | Rapid prototyping, Optomechanic parts | Cast for PDMS chambers |

Strings of plastic are left behind. Possible solutions to overcome “stringing” are increasing the speed at which the extruder moves to reduce the time during which 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” (Fig. 7bv). Tuning a 3D printer to avoid these and other printing artifacts is now easier due to the availability of several 3D models that can be used to optimise specific printing settings. Some tuning models are directed towards tuning a specific aspect, while others are “all-in-one” models that combine multiple features of 3D printing (Fig. 7bvi).

Stereolithography (SLA) is the oldest form of 3D printing (104). As it is a highly versatile and accurate form of 3D printing, although being more expensive, the intricate details it can produce are fairly superior to the FDM. Instead of using thermoplastics like FDM methods, it uses thermoset liquids -in the form of liquid resins- cured by UV light. Here, UV light selectively illuminates a small liquid resin area, triggering initiators and photo-polymerising it via radical polymerization, an exothermic process (105). The polymerisation 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 (105). This process is done in layers but due to the use of resin materials, the material’s physical properties such as tensile strength and flexibility are usually inferior when compared to FDM. To overcome this limitation, material manufacturers are constantly creating new formulations and, in some cases, were able to create resins presenting characteristics that are comparable or even better than FDM materials in terms of flexibility or hardness, heat resistance, 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 moulding. 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 25µm, making the printing process slower than a standard FDM. In recent years, masked SLA (mSLA) printers have reached the consumer market. If the chemical process is similar to the SLA -crosslinking resin using UV light- the way the light is processed is different. 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 (72). 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).

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. Thus, a lot of effort is taken to optimise material composition to increase the printability and print quality, but also to enhance physical properties like strength, flexibility, and biocompatibility (106), or optical properties (107).
Box 2. 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 (108). Parameters such as infill density, layer thickness, orientation, raster angle, and air gaps are essential to provide strength to the printed part (109).

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 formation of voids and improve the mechanical integrity of the structure (110, 111).

Nylon PA presents high chemical resistance, tensile strength, and flexibility. These mechanical properties even improve at higher temperatures (112, 113).

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. (114). 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 (114, 115).

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 (115). 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 (116).

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 extruded properly, resulting in cooling difficulties and proneness to warping (117).

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 found in aircraft, cars, drones and rockets (118) or in biomedical applications (90, 91). Due to higher printing temperatures, specialised printers are required (119).

TPE is a flexible rubber-like combination of elastomers and polymers that are also recyclable. It is highly flexible and soft, with particularly good impact resistance and shock absorption (120).

Composite materials contain supplements such as metals (e.g., copper and iron) or carbon-based elements (graphene or short carbon fibres). Supplementing with iron improves thermal conductivity, storage modulus, and glass transition temperature (121). Short continuous composites show increased rigidity and strength, corrosion resistance, and improved chemical resistance (122, 123). Among these elements, carbon, Kevlar and glass are also used alongside naturally found components such as basalt, jute and bamboo (124, 125).

FDM materials. FDM uses thermoplastic melts dispensed by a heated extrusion print head. 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 fibre reinforced composites (FRC) (126). Given its wide appeal, FDM uses a large selection of thermoplastics commercially.

Common materials include standard plastics used for non-critical functions and engineering plastics that, due to their mechanical properties, are suitable for high mechanical stress work. The most prominent materials are Acrylonitrile Butadiene Styrene (ABS), Polyactic Acid (PLA), Acrylonitrile Styrene Acrylate (Nylon PA), Polyethylene terephthalate (PET), Polyethylene terephthalate Glycol-modified (PETG) Polycarbonates (PC), and polypropylene (PP). 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 costs 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 ”Box 2. FDM-material guide” in the right column.

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 optimisation (9). Since light can only print a layer of limited thickness, multiple light exposures are needed (127).

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 polymerisation. These PIs are usually single molecules that cleave radical fragments when exposed to the light of a specific wavelength (127). Common PIs include phenylphosphine oxide (e.g. Irgacure) (9) and Acril phosphate oxides (e.g., TPO and BAPO) (106). Other methods use two elements: a light-absorbing molecule and a co-initiator (106).

Furthermore, PIs, such as ethyl 4-dimethylaminobenzoate (DMAB) and zinctetraphenylporphyrin (ZnTPP), use visible light to achieve the same effect but display high toxicity (128, 129). 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 are the monomers used to form the 3D object. Originally, they were composed of combinations of diacrylates dissolved in liquid acrylate or methacrylate (9). Many types of monomers are used in commercial and research applications; a few reviews can also be consulted for more in-depth information (9, 131, 132).

Commercially speaking, many different light-curing resins are available with different prices and properties. However, many of these resins are proprietary, making it difficult to understand 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 are helpful in creating mould patterns and provide very high printing detail. Tough resins mimic the mechanical and chemical properties of ABS or PLA, they are often slightly worse than their thermoplastic counterparts and don’t provide the same thermostasis properties. Temperature-resistant resins, on the other hand, are suitable for thermal applications and moulding 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 summarised in Table 2.

Current 3D printing challenges and limitations

3D printing presents ample advantages in customisation, 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 limitations on material choice. 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 important to take the material biocompatibility into account. Many of these materials can be highly toxic to cells and whole organisms (133). Toxicity can be avoided in any case, by using biocompatible post-processing methods if needed. 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.

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 result of the fabrication process. 3D printed parts often carry artefacts depending on the object’s geometry, such as in the case of an insufficient polygon-approximation of curved surfaces (103). Post-processing can often alleviate these defects but good design practices are required to minimise them. Therefore, for the 3D design, it is important 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 certain designs. For example, some designs contain features called “bridges”, requiring horizontal material deposition between two raised points (Fig. 8a). Printing perfectly horizontal bridges is often required. 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 the rate of material extrusion, decreasing nozzle temperature, and decreasing printing speed.

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 deleterious 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 (Fig. 8bi-iv).

An important 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 (134), and this is influenced by the choice of material and technology used, with FDM having void issues more commonly than SLA (134). In the case of 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 (135). Interestingly, this 3D printing flaw can also be exploited by controlling the porosity to develop porous scaffolds that can be employed in tissue engineering applications (136). 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
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 an appropriate understanding of the limitations of the materials to correctly apply them.

| Material | Speed | Low cost | Heat resistance | Chemical resistance | High strength | Flexibility | High detail | Transparency | Bio-compatibility |
|----------|-------|----------|----------------|--------------------|---------------|-------------|-------------|--------------|-----------------|
| ABS      |       |          |                |                    |               |             |             |              |                 |
| PLA      |       |          |                |                    |               |             |             |              |                 |
| PETG     |       |          |                |                    |               |             |             |              |                 |
| PEI, PPSU & PEEK |   |          |                |                    |               |             |             |              |                 |
| Nylon PA |       |          |                |                    |               |             |             |              |                 |
| TPE      |       |          |                |                    |               |             |             |              |                 |
| Standard resin | |         |                |                    |               |             |             |              |                 |
| Clear resin |       |          |                |                    |               |             |             |              |                 |
| Castable resin **)” | |          |                |                    |               |             |             |              |                 |
| Rubber-like resin | |          |                |                    |               |             |             |              |                 |
| Though resin |       |          |                |                    |               |             |             |              |                 |
| Bio-based resin | |          |                |                    |               |             |             |              |                 |
| Thermo resistant resin | |          |                |                    |               |             |             |              |                 |
| Dental resin |       |          |                |                    |               |             |             |              |                 |

*) Can be made biocompatible with post-processing  
**) for mould creation

mechanical behaviour, particularly when vertical tension or compression are exerted on the printed object. This is more common in thermoplastics printed with FDM (137). 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 Acrylonitrile butadiene styrene (ABS) (138). FDM also results in the appearance of layers in the final printed product. This is perhaps not an important factor when the part is not visible, but the exterior parts often need post-processing, such as sintering, to correct this problem (103). For SLA this is usually not an issue.

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 10993. This certification comprises several standards for evaluating medical devices and material biocompatibility to assess and manage the biological risk (139). Polymers in contact with biological tissues undergo degradation due to mechanical or chemical stress, especially in the case of long-term contact. Materials in 3D printing applications for microscopy are generally not expected to undergo long term contact with living tissues. Nonetheless, material toxicity needs to be assessed. This review focuses on 3D printing in microscopy applications and thus, its scope is limited to materials that are in contact with organisms, tissues, and cells during imaging approaches. Notably, these include both the biological specimen and the user. Reviews that deal with long-term exposure of 3D printed materials can also be found as part of biomedical research (140–143).

Given that FDM is the most user-friendly 3D printing technology, it is reasonable to adopt this technology for biological purposes. Previous work has employed materials such as ABS (144), PC (145) and PET (146) 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 (147). On the other hand, photopolymers are often more toxic due to their nature and the potential residues that remain following the photo-curing processes (133). Several studies considered 3D printed objects using SLA technology as toxic when used directly in biological applications without post-processing (148–150). 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 (151). Similarly to FDM-based printed objects, improving biocompatibility requires post-processing. 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 that have been 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 known to be toxic for living organisms (149). Material Residues remaining on the printed objects’ surface can be washed with ethanol, sonication, and sterilised with UV light (149). Another study identified uncured residual monomers in the objects’ surface using HPLC-MS, explaining their high toxicity, and suggested using a combination of residual photopolymer extraction via supercritical CO2 treatment and
Fig. 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.

Environmental impact. The environmental impact of 3D printing processes is still an ongoing topic of discussion within the field (154). To address the environmental impact of 3D printing, three aspects will be discussed in this review: energy consumption, waste management, and air pollution (155). Energy consumption is considered the factor with the largest impact, particularly in mass production (10). Energy consumption depends on material choice, build volume, layer thickness, and printing speed. The energy consumed during 3D printing can be separated into three phases: preheating, printing, and cooling. Current 3D printers are not highly optimised for energy consumption (156). This optimisation lies partially in the often overlooked cooling systems of 3D printers, which mostly rely on electrically powered fans to dissipate heat (157).

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. 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 (158). Recycled PLA or PETG have also started appearing on the market. Another important piece of plastic, usually overlooked when talking about environmental impact, is the spool holders, where the filament is coiled on. As this is a single-use product, some manufacturers have already started using cardboard instead of plastic for spool holders. As the consumer drives the market, a proper choice of recycled filaments and spool holders may guide the market in a more environmentally friendly 3D printing attitude. In addition to this, 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 (155). For FDM, the recycling process is more straightforward. 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 (158).

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 (159). This is particularly hazardous in enclosed environments, and protective clothing and masks are recommended, as 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 compared to traditional manufacturing methods, providing a way to optimise industrial processes. The design of the 3D printed part is also important, as parameters such as layer thickness or printing orientation can demand more energy, requiring fine-tuning to optimise energy consumption as much as possible. Additionally, using “green” or bioelements as 3D printing materials can reduce the printing process’s environmental impact (160). 3D printing is still a young technology and there is ample room for optimisation, particularly in energy consumption and cooling systems, recycling of materials, and emissions. As the technology becomes more standardised, machine design and printing processes are expected to improve.

**Outlook**

Additive manufacturing is revolutionising 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 wide view of the application of commercial printers. 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 are minor setbacks and are expected to improve 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 both in industrial and academic settings.

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**Box 3. 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).

**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 PLA and 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 1. Software Tools", p. 2).

The 3D CAD model is then exported in the STL file format, which encodes the object’s surface geometry but has no information about its colour 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 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. 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 initialise 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 miniaturised 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-lapses 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 materialisation 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 are available, where users actively engage with and benefit from the maker community by sharing their designs, advice and expertise.

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 optimisation of the printing processes, as current 3D printers are not heavily optimised in terms of energy consumption. Plastic waste management is also a matter of standardising recycling processes to take full advantage of discarded printing materials. Pollution also depends on the optimisation 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 in turn will facilitate the accessibility of the technology. Despite current limitations, 3D printing is already enabling unprecedented customisation 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 allows rapid prototyping and design iteration that would be otherwise difficult -if not impossible- with other fabrication technologies.
lar organisation within the tissue. Thus, this approach can only replicate the complex structure and environment of a tissue to a relatively small degree. Bioprinting using bioinks overcomes these limitations by enabling high-precision reconstruction of tissue microarchitectures in a controlled and reproducible manner. For example, a technique called “core-shell bioprinting” allows coaxial printing of two bioinks and ensures a high contact area between the two phases. Taymour et al. used this approach to create a biologically-active 3D in vitro model of the liver microenvironment containing hepatocytes and fibroblasts (164). Furthermore, bioinks have a promising therapeutic potential, namely in the field of wound healing. For example, Nulty et al. (165) incubated a fibrin-based hydrogel containing human umbilical vein endothelial cells to generate a small in vitro vascular network. This prevascularised scaffold was then implanted into mice with critically-sized bone defects, resulting in increased vascularisation and bone formation compared to mice implanted with an immature and disorganized scaffold containing the same cellular components. These implementations showcase the therapeutic potential of 3D printing and the impact that developing this technology can have in biomedical research.

The future and reach of 3D printing. The examples of 3D printing’s applications provided in this review highlight how its emergence revolutionised the manufacturing landscape. 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 accessibility 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-scaled 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 the designs between researchers. Furthermore, the different printing technologies and materials available, coupled with the control over several printing settings/parameters, enable users to manage crucial factors influencing their models’ applicability, such as structural integrity and detail.

The establishment of 3D printing in an increasing number of instances, and the urge to benefit from its advantages, result in the continuous expansion of its current 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 the development of 3D printing technology its own high-impact research field. In this sense, the field of 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 be used to improve current mechanical designs underlying the function of specific microscope parts. In the high degree of movement precision and mechanism miniaturisation that can be achieved with 3D-printed compliant mechanisms make them a promising venue for developments in stage and sample micromovement applications.

Supporting Information
- Movie S1: 3D printed microscope projects (https://youtu.be/X4w4wzvV3JB)
- Movie S2: 3D printed sample manipulation projects (https://youtu.be/p5-FA związaneqVbA)
- Movie S3: "How to" guide for 3D printing (https://youtu.be/4W2CfHyg)
- Supplementary Database: 3D printing database for microscopy (https://doi.org/10.6084/m9.figshare.14579439)

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Extended Author Information
- Mario Del Rosario: @0000-0002-0430-1463, @martiodel
- Hannah S. Hell: @0000-0003-4279-7022, @Hannah_SuperRes
- Afonso Mendes: @0000-0001-7324-555X, @AfonsoMendes92
- Vittorio Saggiono: @0000-0001-7196-602X, @VSaggiono
- Ricardo Henriques: @0000-0002-2043-5234, @RHenriquesLab

Author Contributions
M.D., H.H. and A.M. contributed equally to the preparation of the manuscript, writing the document, preparing figures, tables, and movies. All authors planned and supervised experiments, and received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement No. [101001323] (R.H.), the European Molecular Biology Organization (EMBO) Installation Grant (EMBO-2020-IG-4734) (R.H.) and the Wellcome Trust (203276/Z/16/Z) (R.H.). A.M. would like to acknowledge support from the Integrative Biology and Biomedicine PhD programme from Instituto Gulbenkian de Ciência.

Competing Financial Interests
The authors declare no competing financial interests.

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Mario Del Rosario graduated as a marine biologist from ESPOL (Ecuador) in 2012. He completed a MRes in Biomedical Sciences in the University of Glasgow (UK) in 2014 and stayed for a PhD in the Welcome Centre for Integrative Parasitology (UK) until 2019. During his PhD he worked in the invasion dynamics of Toxoplasma gondii combining parasite work, live imaging and super-resolution microscopy. He is currently a postdoctoral researcher in the Optical Cell Biology Lab at the Instituto Gulbenkian de Ciência (Portugal). He is interested in viral host-pathogen interactions and imaging approaches that reduces cell photodamage during image acquisition.

Hannah S. Heil received her M.Sc. in Nanotechnology from the Julius-Maximilian-University Würzburg (Germany) in 2015. She completed her Ph.D. in at the Graduate School of Life Science of the University of Würzburg in 2020 where she developed advanced imaging tools by combining biophotonics and super-resolution microscopy. She is currently a postdoctoral researcher in the Optical Cell Biology Lab at the Instituto Gulbenkian de Ciência (Portugal). Her current research interests include the development of automated and intelligent super-resolution microscopy to study host pathogen interactions during viral infection.

Afonso Mendes graduated in Biology at FCUL (2017, Portugal) and obtained a Master's degree in Biomedical Research at NOVA Medical School (2019, Portugal). He was selected for the IBB PhD Programme at IGC (2020, Portugal), where his passion for viruses combined with a growing desire to explore computational analysis and technology development led him to join Dr. Ricardo Henriques' laboratory as a PhD student.
Dr. Vittorio Saggiomo obtained his MSc. degree in Organic Chemistry in 2007 at the University Federico II of Naples (Italy). He moved to Kiel (Germany) for pursuing his Ph.D. working on Dynamic Combinatorial Chemistry. In 2012, he started as postdoc at the university of Groningen (the Netherlands) in the field of Systems Chemistry. In 2013 he did a second postdoc at Wageningen University (the Netherlands). He is currently an Assistant Professor at the same University in the BioNanoTechnology group. His interests and expertise spread from organic chemistry to Open Technologies and 3D printing in microfabrication.

Prof. Ricardo Henriques graduated from Physics Engineering at FCUL (2006, Portugal). He carried out PhD research in optical physics and biophysics at IMM (2008, Portugal), CSIR (South Africa, 2008) and Institute Pasteur (France, 2008). He did a short postdoc in Institute Pasteur (2011, France), started a research group at UCL (2013, UK), and then a second laboratory at the Francis Crick Institute (2017, UK). Became full professor at UCL (2018, UK) and recently started moving his laboratory to IGC (2020, Portugal) where he applies optical physics, machine-learning and cell biology to study viral infection.