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Additive manufacturing for lab applications in environmental sciences: Pushing the boundaries of rapid prototyping

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ABSTRACT

Fused Deposition Modeling (FDM), better known as 3D printing, has revolutionized modern manufacturing processes and the ever-increasing use of 3D printers is popular not least because of the wide range of materials available for printing. When applying the FDM process to the development of prototypes, it is possible to go from an idea to a first iteration of the product within a few hours, and from an initial concept to a final product within a few days depending on the complexity of the desired structure. We applied FDM-related open-source 3D software and a 3D printer to produce parts for devices being applied in wood anatomy and dendroecology. In this paper, we present the basic requirements for prototyping by showing detailed examples of new devices developed and produced using a 3D printer and related modeling software.

1. Introduction

Wood anatomical studies typically describe the anatomical structures of plants and their variability e.g., across different species or genera (Antonelli et al., 2019; Selvi et al., 2019). For this purpose, small micro sections (approx. 1 cm) in transverse, tangential, and radial directions are sufficient to identify the features of interest. In recent years, the application of wood anatomical techniques in dendroecological studies has become increasingly important (Gärtner et al., 2015a). Detailed analyzes of cell structures and their variation within the rings of woody plants allow for new insights into a plant's reaction to environmental changes on an intra annual scale (Bräuning et al., 2016; Gärtner and Farahat, 2021). In extension of the analysis of tree-ring widths intra annual anatomical parameters open opportunities for new research questions focusing on reconstructing environmental changes further back in time. Since these upcoming research questions no longer focused on single rings, but required the analysis of longer time series, this resulted in the need to cut bigger sections of high quality. This was supported by the development of new microtomes developed especially for cutting bigger specimens (Gärtner et al., 2015b, 2015c). The fact that anatomical applications have more or less evolved to a standard in dendroecological research is thanks to the innovative strength of many researchers and technicians in the field developing new techniques for sample preparation (Campbell et al., 2011; Nakai et al., 2018), stabilization (Schneider and Gärtner, 2013), or embedding (Balzano et al., 2021; Deslauriers et al., 2015). Numerous innovations have been proposed by the dendro-community, such as the use of cable guides or wavy cardboard to fix increment cores below a binocular, or special holders for long micro slides to allow the capture of microscopic images (Klisz et al., 2018). Nevertheless, any further development of such holders (or other such innovations) typically requires technical knowhow, mechanical skills, and specialized machines for metal and/ or wood processing. When mechanical requirements such as load bearing or moveable parts are required, further development of new or existing tools is mostly impossible without the support of an experienced mechanic or carpenter and the appropriate equipment.

The same problem refers to the daily work of many lab and field technicians are familiar with these problems. Most if not all technicians experience a moment in which they identify the need for a specific adapter, holder, or other specialized tools or parts. In most cases, technicians spend hours scouring the web looking for parts that simply are not for sale anywhere. The same is true of parts for the older equipment used in labs all over the world. If part of a device is broken or lost, one needs to find and order replacement parts (if available) or find a mechanic capable of producing the (in most cases metal) replacement part. Involving a specialist from an external, non-scientific, and/or profit-oriented company is time consuming and expensive. As a result, many good ideas fizzle out because there is simply no money to engage a specialist to pursue them further. Creating a prototype in metal form is no longer impossible, as companies that do small-scale metal

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manufacturing now exist. However, as the name implies, a prototype is not a final product. Prototyping involves creating a first draft and then iterating until all the problems are solved. In the case of a metal product, this involves investing a considerable amount of time in explaining the requirements to the mechanics, as well as a considerable amount of time, material, and money to create said prototypes until the final iteration is achieved.

"Additive Manufacturing" (AM) offers a possible solution. The most common and low-cost method within AM is extrusion-based "Fused Deposition Modeling (FDM), where a spool of thermoplastic material in wire form (i.e., filament) is used as feedstock (Gao et al., 2020). This method is commonly known as "3D printing" (Ngo et al., 2018). In 3D printing, a printer places semi-molten material on a bed, forming an object layer by layer until it reaches its full dimension (Jayanth et al., 2021). AM has revolutionized modern manufacturing processes; the ever-increasing popularity of FDM printers is due in large part to the ever-increasing range of materials available for printing (Lee et al., 2017), as e.g., new flexible filament, owing optical effects as color changing, wood imitation, metal imitation or fluorescence, or filaments with improved physical properties like food safe, heat resistant, low friction, or dissolvable characteristics (Li et al., 2019; Singh et al., 2017). Thanks to the ongoing development of techniques and technologies, it is now possible to even print metals and ceramics (Zhang et al., 2018). Once a niche product, 3D printers have significantly improved and further developed for several years now. These printers are now found in a variety of applications in both the commercial and private sectors (Evans, 2012). The use of printers for private and hobbyist applications in particular has led to an immense growth in the market; nowadays, 3D printers are available (and affordable) for educational (Szulżyk-Cieplak et al., 2014), biomedical (Kamio et al., 2018), and other scientific applications (Jones, 2012).

When applying the FDM process to the development of prototypes, it is possible to go from an idea to a first iteration of the product within a few hours, and from an initial concept to a final product within a few days depending on the complexity of the desired structure. With these applications in mind, we used FDM-related open-source 3D software and a 3D printer to produce parts for devices being applied in wood anatomy and dendroecology. In this study, we present the basic requirements for prototyping by showing detailed examples of new devices developed and produced using a 3D printer and related modeling software.

2. Printer

Buyers should consider several things before purchasing one of the numerous 3D printers available on the market. One of the most important considerations is health, as there are differences in the aerosol (nanoparticle) emission rates of different printers (Mendes et al., 2017), as well as of the materials used for printing.

Because we intended to stay in a medium price segment (below 1000 Euro), we decided to use the "Original Prusa i3 MK3S+" printer (Prusa Research A.S.; Czech Republic). We used the printer in combination with open-source software (PrusaSlicer) that enables the export of files generated by (any) modeling software to the 3D printer.

3. Modeling software

The foundation for all objects printed in three dimensions is a digital 3D representation of the object, i.e., a model. Numerous internet platforms offer free, ready-to-use models of many fairly common objects. One of the most popular platforms in this regard is "thingiverse.com", a website dedicated to collecting and sharing user-produced digital design data. Nevertheless, the models available seldom correspond to objects needed when developing new devices. As a consequence, these models have to be custom designed using modeling software, of which many different types exist, no matter if license based or freeware. The modeling approaches of the various software packages differ widely: some have a more art- and sculpture-inspired design approach, some allow the user to drag and drop modifiable geometric shapes, and others are based on sketches or CAD (Computer Aided Design). The choice of the right software depends on the user's preferences and skills. In our lab, we use OpenSCAD, the most widely used scripting tool for parametric modeling of open-source labware (Gohde and Kintel, 2021; Nilsiam and Pearce, 2017). OpenSCAD is a text-based modeling software based on CAD and math, in which text-based commands construct the objects of interest step-by-step. The advantage of this approach is that all parts of the design are precise in position and size. The design of simple objects might take an experienced operator only a few minutes; more complex designs should not take much more than a few hours.

4. Material

The most commonly used filament in the process of 3D printing is polylactic acid (PLA). PLA is a synthetic polymer used to produce plastic that is obtained from regenerative sources (such as corn starch). PLA is therefore considered to be a biocompatible raw material. PLA filaments used for 3D printing are in most cases PLA "blends", the basic structures of which are enriched with additives to improve printing and appearance (Jayanth et al., 2021). Another widespread filament is an acrylonitrile-butadiene-styrene (ABS)-based plastic. As a synthetic polymer, ABS is one of the most common plastics worldwide and is particularly resistant to oils, fats, and high temperatures. Probably the most important properties of ABS are its rigidity, toughness, and strength (Khabia and Jain, 2020). However, ABS is only moderately UV-resistant. For this reason, objects that are to be used outdoors (e.g., temperature loggers) should be made with acrylic ester-styrene-acrylonitrile (ASA) filaments, which are extremely weather-resistant. If transparent objects are needed, a PETG filament (glycol-modified polyethylene terephthalate) is the right choice, since PETG is characterized by its particularly high transparency and low viscosity. PETG is a thermoplastic that is familiar to most people in the form of PET bottles.

As of 2022, a spool of good to premium quality filament such as PLA, ABS, or PETG cost about 25€ per Kg. Given the fact that printing most objects (such as the camera holder shown in Fig. 1) only requires a few grams of the material, 3D printing can be a very economical option. The economic use of printing filament is due to the fact that objects are not printed as full, solid, massive blocks, but are instead printed using a so-called gyroid infill (Fig. 1). The gyroid is one of the few 3D structures that gives equal strength in all directions, providing great support to the structure. Gyroids are also printed relatively fast, save material, and don't cross themselves at one layer. In case greater stability is required, the resulting structure can be infilled with resin or other liquids after printing (Silva et al., 2021).

5. Examples of objects designed for applications in wood anatomy

5.1. Microscope adapter for axis alignment of common slides

To measure the anatomical parameters of a given sample, digital images of the cell structures of interest are required. Laboratories that deal with a huge number of micro slides on a daily basis might have automated slide scanners such as the Zeiss Axio Scan.Z1, which enable the digitization of up to 100 micro slides in a single run. Most laboratories, however, use microscopes equipped with cameras to digitize the samples manually. Because studies of wood anatomy are increasingly focused on longer time series, the micro sections cut are getting longer (e.g., sections of increment cores). The single images shot along the length of the core are then stitched together using specialized software, resulting in a panorama image of the entire section. These long sections are rarely aligned parallel to the slide length; in most cases, the sections rotate during embedding, or are aligned obliquely to allow for more than

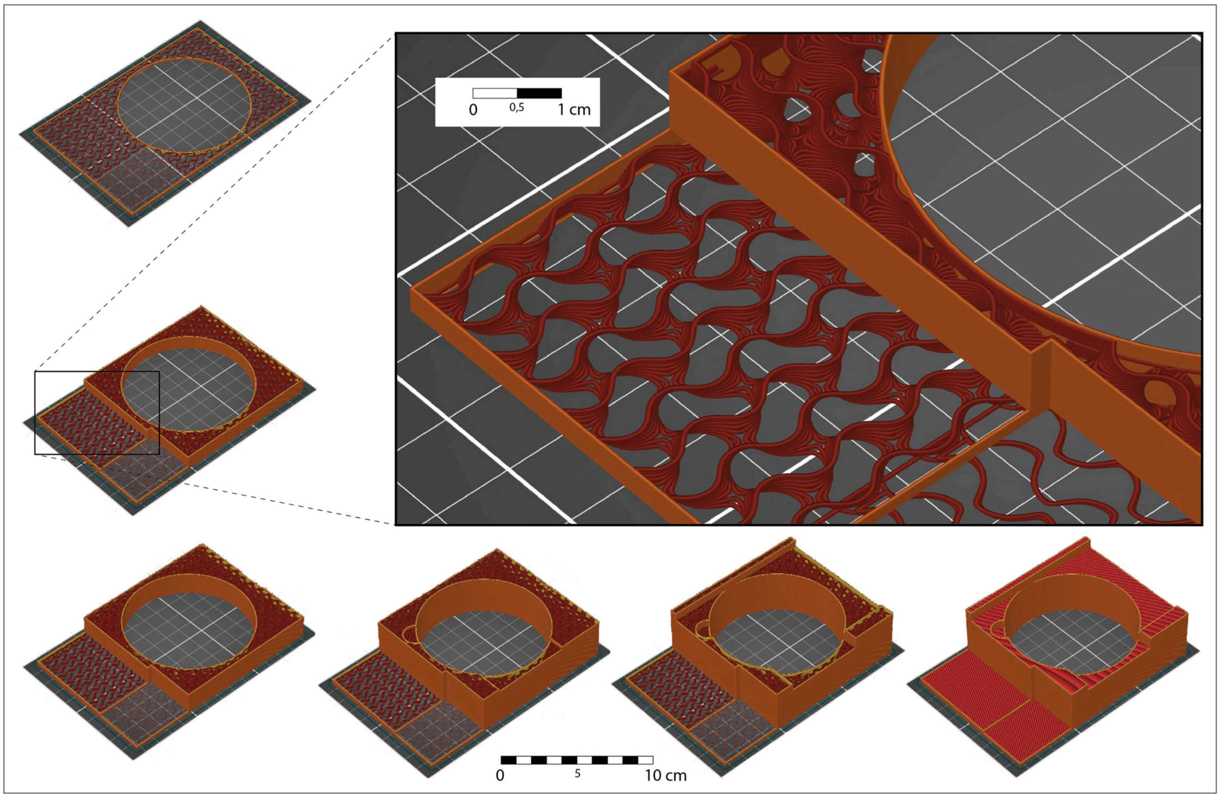

Fig. 1. Illustration of single layers of a camera holder designed with OpenSCAD. The magnified part in the upper right corner of the figure displays details of the gyroid infill used to stabilize the object while saving weight.

one section to be fixed below the cover glass. If an operator intends to take pictures over the entire length of an oblique section, one has to move the section below the objective along the x- AND the y-axis (sideways and up/down). Because this procedure is rather complicated and time consuming, the resulting panorama images are seldom straight (Fig. 2). In most cases, this occurs because the microscope table cannot be turned at an angle to allow the operator to move the section along its own longitudinal axis. Even if microscope tables could be turned, one of the axes would also be turned, resulting in the same problem.

To solve this problem, we designed a ring holder. To use the holder, the operator places the slides in a circular frame, which is then fixed to the microscope table in the same way as the original slide holder (Fig. 3). This simple ring can now be moved along the x- and y-axes of the microscope in the same manner as one would move the original holder. However, but the glass slide placed in the ring can now be rotated 360° so that the section of interest can be aligned and moved along one axis without requiring further correction while shooting the single images.

The dimensions of the screw holes at one end of the ring adapter can be adjusted to any type of microscope table. Simply measure the dimensions and position of the microscope screws that fix the original

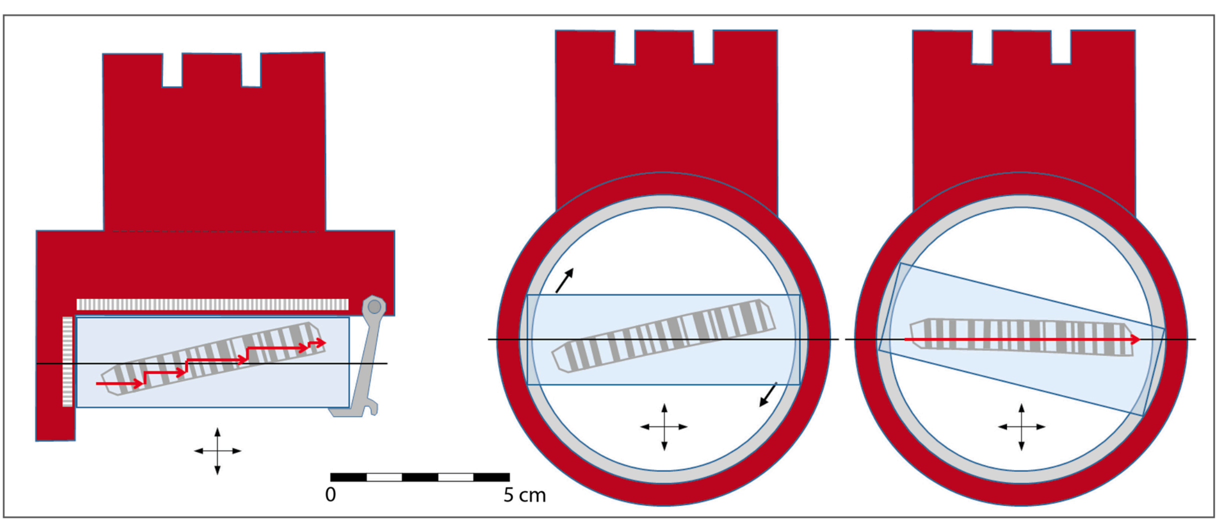

Fig. 2. The traditional slide holder of a microscope (left) requires frequent correction of the pathway (red arrows) to create a panorama image. The ring adapter (middle and right) enables the operator to align the object such that a single straight path results (red arrow in the right image). The ring adapter is designed for standard glass slides (76,2 × 25,4 mm) as used in microscopy.

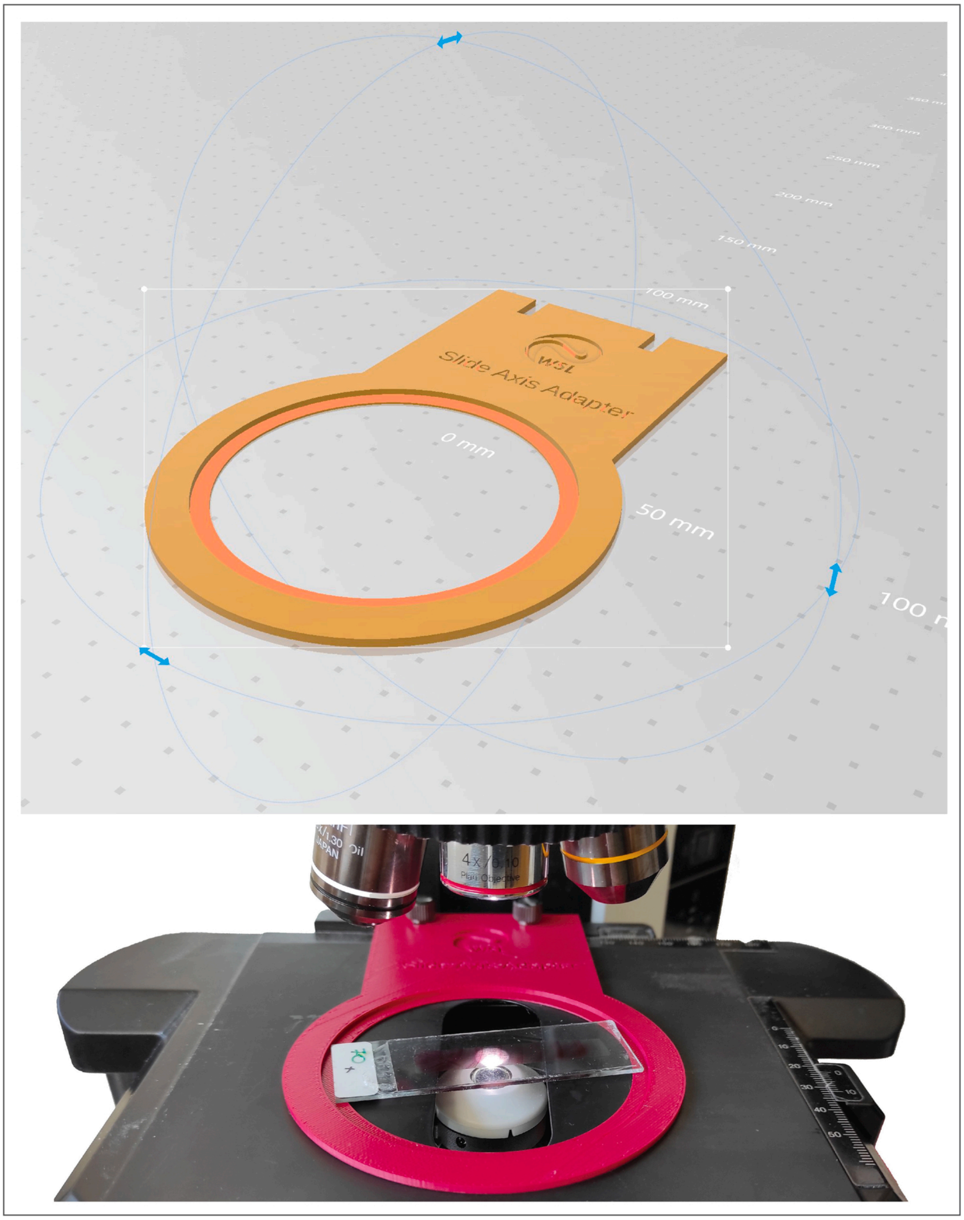

Fig. 3. 3D model of the slide axis adapter (top) and the printed version (bottom) mounted on a microscope table.

slide holder and enter the data into the 3D model before printing.

5.2. *Microscope adapter for long slides (non-standard glass format)*

Another common problem regarding the use of microscopes to prepare digital images of micro sections arises when non-standardized slides are used. Common microscopes are equipped with holders for standardized glass slides (76,2 × 25,4 mm). In dendro-anatomical studies, it is becoming increasingly common to cut micro sections from entire increment cores (Gärtner and Nievergelt, 2010; Gärtner et al., 2015b; Ivanova et al., 2015). These micro sections need to be processed and stabilized on special glass slides that are up to 40 cm long. When developing the method for cutting micro sections of this dimension, Gärtner et al. (2015b) also proposed creating a holder for these slides made out of cardboard. Because cardboard holders are not very stable, Klisz et al. (2018) developed a more sophisticated but expensive long slide holder that allows the user to precisely position the slide below a microscope.

3D printers now allow for the creation of very stable slide holders based on the initial cardboard model (Gärtner et al., 2015b). The stability of the printed holders allows the user to quite precisely move the slide sideways below the objective of the microscope (Fig. 4). Because the micro sections of an entire core are positioned more or less straight along the glass slide, the corrections needed to create images of the core are minimal. However, if a core breaks into pieces during the embedding process, it may still be necessary to turn these long slides to create a straight image line (see Fig. 2). To enable this functionality, the model of the holder can easily be adapted by placing a joint between the holder itself and the part that is fixed to the microscope table. Applying such changes to the 3D-model is very easy and can be realized at any time. The same is true for the dimensions of the recesses for the screws on the microscope table; they can be readily adapted to meet the user's needs.

5.3. *Storage frame for microtome blades or blade holders*

The advent of specialized microtomes for cutting wooden specimens has led a widespread application of wood anatomical techniques in dendroecology (Gärtner and Nievergelt, 2010; Gärtner et al., 2014,

Fig. 4. 3D model of the long slide holder (top) and the printed holder (bottom) mounted on a microscope table.

2015c). These microtomes are standard equipment in many labs, no matter if operated with full microtome blades or holders for disposable blades. For all microtomes, securing the blade when the microtome is not in use, even if only for a short period of time, is a challenge. Especially when using a device with disposable blades, it is frequently necessary to remove the blade holder from the microtome right after installing a new blade, e.g., to avoid injuries when changing the sample in the sample holder. This is faster and easier than removing the blade from the holder. Leaving the blade or the blade holder in the microtome is as dangerous as taking the blade or the holder out and placing it on the table next to the microtome. The user risks cutting themselves on the microtome blade when cleaning the microtome before or after changing samples, as well as when the blade is picked up without sufficient care.

We found a simple solution for this problem by printing a storage frame for blade holders, which can also be adapted to common blades (Fig. 5). The blade can be placed in the frame with the cutting edge facing down. The same is true for the blade holder when a disposable blade is used. With this storage frame, you avoid potential damages to the blade itself and the blade or the holder can easily be handled by only touching the blunt side.

5.4. *Core holder for tangential sectioning*

When focusing on wood anatomical studies in Dendroecology, the ability to view tree rings in their transversal plane (cross section) is important for developing long time series. If single cell rows are clearly visible, a transverse view also provides intra-annual resolution. For intra-annual isotope studies, single tree rings need to be cut in smaller pieces along the growing direction of the rings. To achieve this, small tangential sections need to be cut or split off an increment core using a scalpel or razor blade beneath a binocular (Szymczak et al., 2019). This technique is sufficient for separating annual rings or a few blocks out of wider rings, but is not precise enough to split off pieces of the same thickness within the rings of an entire increment core. A perfect solution for this problem would be to use a microtome to cut tangential sections of a defined thickness (e.g., 30 μm). Unfortunately, microtomes do not have holders for fixing increment cores in a way that allows the core to be cut tangentially without first splitting it into smaller (1–2 cm) pieces. This mostly results in a selective loss of material along the core.

To avoid the loss of material that results from splitting a core into smaller sections, we designed a special holder that allows the user to fix

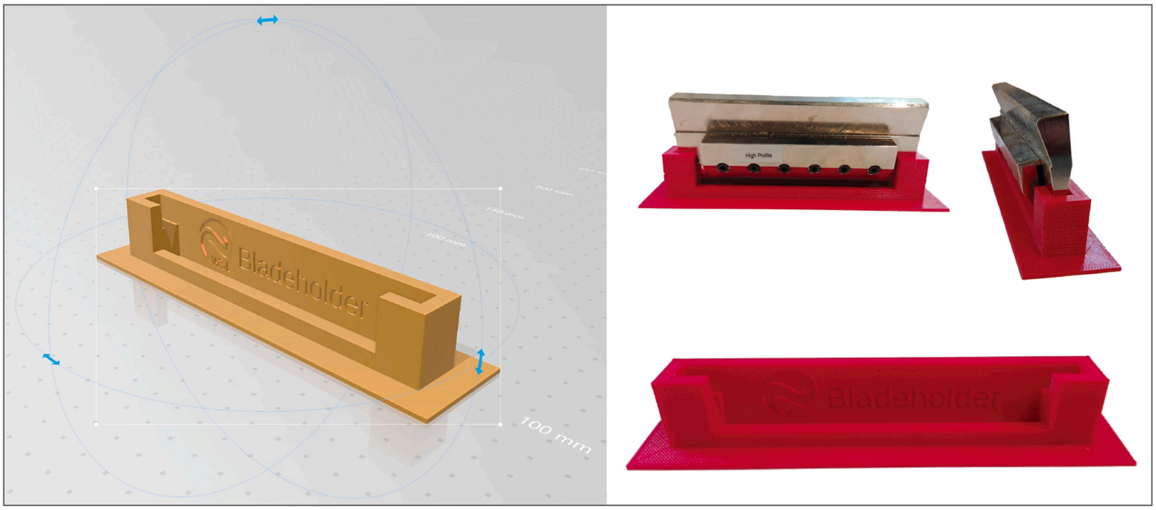

Fig. 5. 3D model of the storage frame (left) and the printed version with and without blade holder (right).

increment core pieces of up to 6 cm in length longitudinally in a microtome (Fig. 6). This holder contains two holes, one for the core and one with threads for a screw that is used to fasten the core while cutting. As soon as the core is cut such that it is flush with the edge of the holder, the screw can be loosened and the core moved to a new position to continue cutting tangential sections. This holder can easily be adapted before printing to any core diameter.

5.5. Potential problems in creating models and transferring them to a printer

Although 3D printing is cheap and efficient, designing an object and printing it to the correct scale can be challenging. Many of the free or affordable programs are designed for artists and therefore possess mesh and freeform tools that are not very useful for designing mechanically accurate objects. In this regard, CAD programs are optimally suited for mechanical design. However, not all of these programs allow the export of the model in the format required for 3D printing. Dimensional accuracy is another challenge, as calibration of the dimensions of the object to be printed can be difficult.

All models presented above were designed using OpenSCAD, a free CAD program capable of exporting the *.stl files required for most printers. Another advantage of OpenSCAD is that the units used for modeling correspond to millimeters. Thus, depending on the material used, the models can be designed and printed 1:1 without any further scale adaption. It should be noted, however, that the material itself might cause problems in printing the object to scale. Some materials tend to shrink significantly while printing, requiring corrections to the scale of the model and sometimes the printer environment and settings.

Depending on the mechanical stress exerted on the printed object

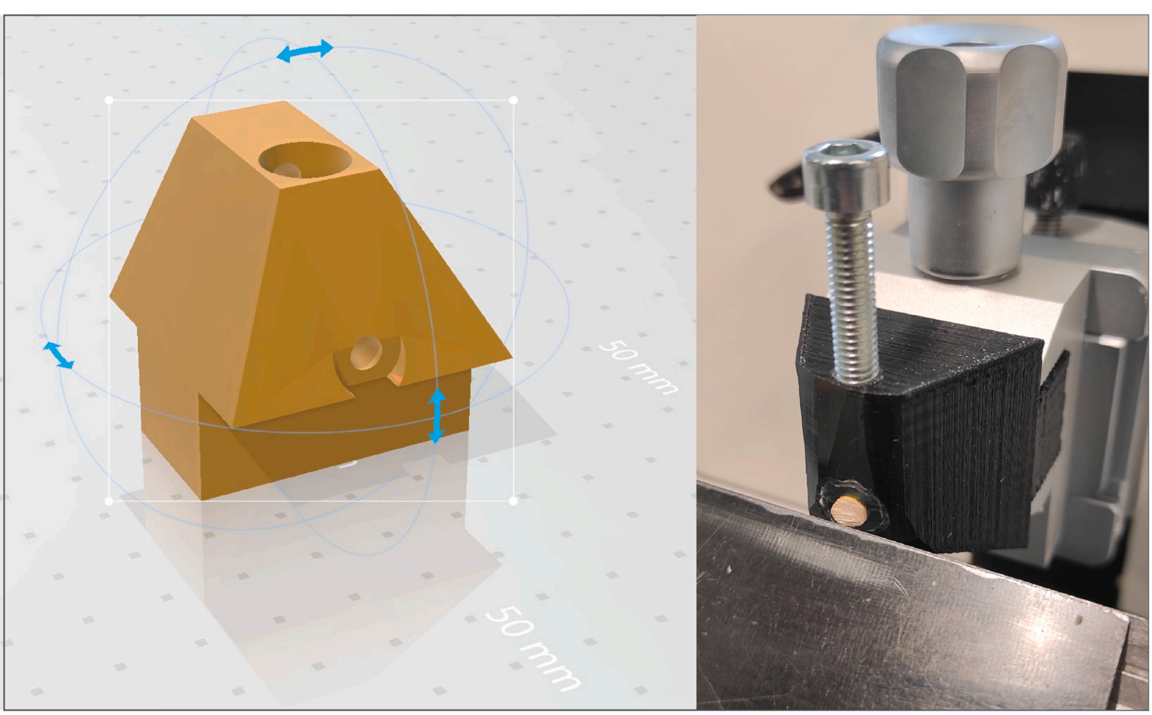

Fig. 6. Microtome core adapter model (L) and the printed version (R) attached to a rotational microtome. The screw used for this example might be a bit oversized, but is efficient due to its large contact area with the core.

while in use, a suitable material should be chosen for printing. The objects presented in Figs. 1–5 were printed with PLA because they do not experience significant mechanical stress. In contrast, the core holder presented in Fig. 6 was printed using PETG. Both the material used and the amount and type of infill used influence the mechanical properties of the object. It is therefore very important to select materials appropriate to the intended use of the printed object. As a rule of thumb, the more mechanical stress the model has to withstand, the more infill is needed. In this regard, the object can be printed as a massive block (very long printing time and high material usage) or as a gyroid infill (faster printing and lower material usage; Fig. 1). In the case of a gyroid infill, the object can later be filled with e.g., epoxy resin to reach a very high stability. For most of the objects presented above, PLA was of sufficient strength. The only exception was the core holder (Fig. 6), which was printed with PETG.

6. Conclusion

Whether they are used for rapid prototyping or to repair broken pieces of equipment in the lab, 3D printers are invaluable tools for efficiently solving lab-related problems. These printers have evolved from gadgets to enormously helpful tools that can be applied by everybody willing to invest some time in getting familiar with the software required for modeling. They enable users to quickly realize an idea at low cost by developing a model, printing the result, and iterating until the final goal is achieved. Although 3D printers are not well suited for mass production in a lab, they are invaluable for producing specific parts used for one machine, workflow, or experiment. The diversity of available filament types and processing options allows for the printing of almost anything. In addition, there is a huge community-driven effort to further improve 3D printers and related technologies. For numerous potential ideas and problems, other users have likely tested comparable things before and are willing to share their knowledge and experience. In summary, the authors see a huge potential for 3D printing technologies to be applied in the lab. Even if the final product is not as durable as a metal part (if such is even needed), it can easily be re-printed in case of a failure. Printed objects can also be used as blueprints for pieces that require a more traditional fabrication method.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Data Availability

Data of 3D-Models will be made available on request.

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