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Rapid, simple and inexpensive production of custom 3D printed equipment for large-volume fluorescence microscopy

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ABSTRACT

The cost of 3D printing has reduced dramatically over the last few years and is now within reach of many scientific laboratories. This work presents an example of how 3D printing can be applied to the development of custom laboratory equipment that is specifically adapted for use with the novel brain tissue clearing technique, CLARITY. A simple, freely available online software tool was used, along with consumer-grade equipment, to produce a brain slicing chamber and a combined antibody staining and imaging chamber. Using standard 3D printers we were able to produce research-grade parts in an iterative manner at a fraction of the cost of commercial equipment. 3D printing provides a reproducible, flexible, simple and cost-effective method for researchers to produce the equipment needed to quickly adopt new methods.

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1. Introduction

As research questions in neuroscience, and biomedical science in general, become more complex, so too do the techniques involved. Methodological advances also appear more frequently, leading to a greater rush for research groups to utilise these within their field. However, each novel method brings its own problems in the equipment that is required. A good example of this is tissue clearing (Höckendorf et al., 2014; Kim et al., 2013), the process by which biological tissue is rendered transparent to allow for light microscopic investigation of large volumes of brain tissue. A number of tissue-clearing methods have been recently developed (Chung et al., 2013; Hama et al., 2011; Ke et al., 2013; Kuwajima et al., 2013; Susaki et al., 2014), but the common factor amongst them is that the volumes of tissue involved are orders of magnitude greater than traditional histology, requiring custom laboratory equipment for both the handling and imaging of samples.

Laboratory equipment for these novel techniques can be produced in a number of ways. Commercial manufacturers will develop equipment once experimental techniques have become commonplace, but this is often too late for those researchers wishing to quickly adopt a method and are frequently prohibitively expensive. In addition, these products are often "generic" in nature, so they may fail to precisely provide the desired function, delaying further innovation. Alternatively, custom equipment can be constructed using conventional manufacturing techniques: additive (e.g. welding), net shape (e.g. injection moulding) and subtractive (e.g. machining). However, these methods are expensive and require specialist equipment and training, which is beyond many research groups. External collaborators or contractors may provide this equipment and knowledge, but this can be at the expense of speed, which is required for the rapid prototyping of custom parts.

Layered, additive manufacturing (3D printing) overcomes these problems, allowing for rapid, simple and inexpensive prototyping of custom parts for research. The concept has existed for some time (Hull, 1986), but until recently it has remained expensive and complicated, as is often the case with manufacturing techniques. One method of 3D printing is fused deposition modelling (FDM), (Crump, 1989), which has seen a dramatic decrease in the cost of individual printers and is now readily available to the consumer market. FDM printers are available in either kit form or fully assembled for between $300 and $5000 depending on the

Abbreviations: ABS, acrylonitrile butadiene styrene; PLA, polylactic acid; FDM, fused deposition modelling; SDS, sodium dodecyl sulphate; PFA, paraformaldehyde; STL, stereolithography.

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Table 1
3D printing parameters.

Model	Brain slicing matrix	Staining/imaging chamber
Printer	MakerBot Replicator Mini	MakerBot Replicator 2X
Filament	PLA	ABS
Supports	No	Yes
Raft	Yes	Yes
Infill (%)	10	15
Shells	2	2
Layer height (mm)	0.2	0.1
Extruder temperature (°C)	230	230
Build plate temperature (°C)	N/A	120
Extruder speed while extruding (mm/s)	90	90
Extruder speed while travelling (mm/s)	150	150
Model weight (g)	24.55	12.19
Model cost ($, approx.)	0.69	0.34
Print time (hours, approx.)	3	2

specifications of the machine. These printers work by melting a plastic filament and depositing a layer of material onto a moveable platform. The platform then moves vertically away from the printing head to allow the next layer to be deposited onto the existing layers, allowing a 3D object to be generated. In addition to the low cost, while previously 3D printing required in-depth computer aided design knowledge, most simple parts can now be developed with simple and freely-available tools.

The aim of this study was to explore the use of freely-available software, along with inexpensive consumer grade FDM printers, to produce custom equipment required for a novel tissue clearing method: passive CLARITY (Chung et al., 2013; Tomer et al., 2014).

2. Materials and methods

2.1. Materials

Polylactic acid (PLA) and acrylonitrile butadiene styrene (ABS) filaments were purchased from 3D FilaPrint (UK); 40% acrylamide and 2% bis-acrylamide solutions from Bio-Rad (UK); 16% paraformaldehyde (PFA) solution from Alfa Aesar (UK); VA-044 photoinitiator from Wako Chemicals (Germany); agarose from Roche (UK); sodium dodecyl sulfate (SDS), boric acid and sodium hydroxide (NaOH) from Sigma–Aldrich (UK); phosphate buffered saline (PBS) tablets and glycerol from Fisher Scientific (UK).

2.2. Design software

All models were designed using Tinkercad software (www.tinkercad.com) and exported as STL (STereoLithography) files to MakerBot Desktop printers for printing. Final versions of the STL files are available as Supplementary materials.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2015.03.042.

2.3. 3D printing

All models were produced using either a MakerBot Replicator Mini (using PLA) or a MakerBot Replicator 2X (using ABS) purchased from MakerBot Industries, LLC. All printing parameters are shown in Table 1. Vernier callipers were used to verify the model dimensions after printing.

2.4. Clearing

Adult (C57BL/6) mice were perfused with PBS and brain tissue was fixed with 4% PFA for 24 h. Following this, whole brains were prepared according to the passive CLARITY protocol (Tomer et al., 2014). Each brain was incubated in 40 ml of hydrogel solution (4% acrylamide, 0.05% bis-acrylamide, 0.25% VA-044 and 4% PFA in PBS) at 4 °C for 10 days. The hydrogel was degassed using a vacuum pump, a desiccation chamber and nitrogen gas before polymerisation at 37 °C for 2 h. The brain was removed from the excess hydrogel and embedded in a 6% agarose solution for sectioning. After sectioning, the tissue was cleared in a 4% SDS solution in sodium borate buffer (pH 8.5 with NaOH). Tissue sections were cleared at 37 °C with shaking, and the clearing buffer was replaced weekly until the slice could be placed over printed text without visible distortion of the letters. Prior to imaging, samples were incubated in refractive index (RI) matching solution (85% glycerol) for 24 h.

3. Results and discussion

3.1. Brain slicing matrix

To achieve optimal tissue clearance and antibody staining using the passive CLARITY protocol (Tomer et al., 2014) mouse brain tissue is sectioned before clearing. Sectioning also complements the use of more readily-available microscopy methods (confocal and multiphoton imaging, rather than single-plane illumination microscopy). Traditional rodent-brain matrices for sectioning tissue are commercially available, but these are expensive (~$200 for a single acrylic matrix and $350–$600 for a stainless steel matrix). Further, each matrix is designed for brains of a defined species and age, which significantly limits the flexibility of

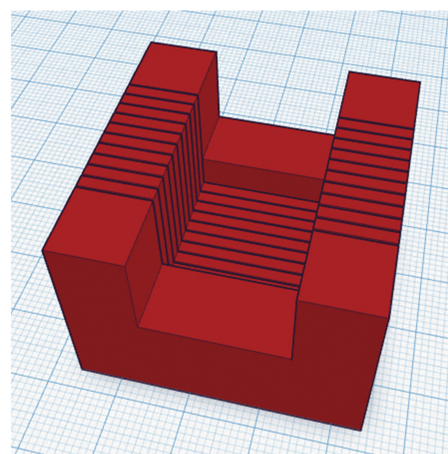

Fig. 1. Initial brain slicing matrix design.

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