Sep 05, 2024 Version 3

Mod3D Live Cell Chambers and holders 3D printing and Assembly V.3

PLOS One

Peer-reviewed method

DOI
dx.doi.org/10.17504/protocols.io.n92ldz587v5b/v3

Ray Truant[1,2], Siobhan Goss[1,2], Siobhan Goss[1,2], Kaitlyn Neuman[1,2], Christina Yi Peng[1,2], Celeste Suart[1,2], Nola Begeja[1,2], Max Davide Truant[1,2]

[1]Department of Biochemistry and Biomedical Sciences, McMaster University. Hamilton, Ontario, Canada, L8N3Z5; [2]Center for Advanced Light Microscopy (CALM), McMaster University.

Ray Truant: Corresponding;
Siobhan Goss: Co-first
Siobhan Goss: Co-first
Max Davide Truant: Auther added for development of 2024 version 2.0

PLOS ONE Lab Protocols Spotlight series

Ray Truant
McMaster University

DOI: dx.doi.org/10.17504/protocols.io.n92ldz587v5b/v3

External link: https://doi.org/10.1371/journal.pone.0269345

Protocol Citation: Ray Truant, Siobhan Goss, Siobhan Goss, Kaitlyn Neuman, Christina Yi Peng, Celeste Suart, Nola Begeja, Max Davide Truant 2024. Mod3D Live Cell Chambers and holders 3D printing and Assembly. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.n92ldz587v5b/v3**Version created by **Ray Truant**

Manuscript citation:
Goss S, Barba Bazan C, Neuman K, Peng C, Begeja N, et al. (2022) Mod3D: A low-cost, flexible modular system of live-cell microscopy chambers and holders. PLOS ONE 17(6): e0269345. https://doi.org/10.1371/journal.pone.0269345

Protocol status: In development
We are still developing and optimizing this protocol

Created: July 31, 2023

Last Modified: September 05, 2024

Protocol Integer ID: 107014

Abstract

Live-cell microscopy imaging typically involves the use of high-quality glass-bottom chambers that allow cell culture, gaseous buffer exchange and optical properties suitable for microscopy applications. However, commercial sources of these chambers can add significant annual costs to cell biology laboratories. Consumer products in three-dimensional printing technology, for both Filament Deposition Modeling (FDM) and Masked Stereo Lithography (MSLA), have resulted in more biomedical research labs adopting the use of these devices for prototyping and manufacturing of lab plastic-based items, but rarely consumables. Here we describe a modular, live-cell chamber with multiple design options that can be mixed per experiment. Single reusable carriers and the use of biodegradable plastics, in a hybrid of FDM and MSLA manufacturing methods, reduce plastic waste. The system is easy to adapt to bespoke designs, with concept-to-prototype in a single day, offers significant cost savings to the users over commercial sources, and no loss in dimensional quality or reliability.

(The **last step** in this version contains a supplemental video with extra context and tips, as part of the protocols.io Spotlight series, featuring conversations with protocol authors.)

1 For FDM prints of holders, print at 20% grid infill with a layer height of 0.16 mm on either a Creality Ender 3 or a CR10 printer (Creality, Shenzhen, China) or similar FDM printer. Poly lactic acid (PLA) or Polyethylene terephthalate glycol (PETG) 1.75mm filament should be used. Acrylonitrile butadiene styrene (ABS) is not recommended as warping is an issue. Print nozzle is 0.4mm size or less and should be 195-205C, with heated bed at 60C, but should be optimized for the plastic being used.

2 For chamber holder tops and bottoms, include a >6mm brim on the bottom to ensure the print remains flat. A thin layer of polyvinyl alcohol (PVA) glue stick on the printer bed can be used to promote adhesion and prevent any warping.

3 To minimize warping, the print should not be removed from the heated print bed until cooled to room temperature.

4 Any PVA glue residue can be removed with a warm water wash.

5 Within the print, glue four neodymium 5x1mm magnets into place on each top and bottom half of the chamber holder with polyurethane or cyanoacrylate glue. Make sure to check that magnet polarity is correct to allow clamping and not repulsion. Allow glue to cure as per glue manufacturer instructions. Chamber printing (MSLA)

6 Use an Anycubic Photon printer (Anycubic, Shenzhen, China), or similar, with eSun PLA biocompatible resin LC1001 (Shenzhen eSUN Industrial Co.,China) using the standard settings for that printer located in the Chitubox software profile, with the exception of a 160 second first later exposure and a 30 second per subsequent layer exposure to ensure inverted print adhesion to platform. Glue surface should be the top or final layer of the print. Make sure the anti-aliasing option is NOT used in the mask profile.

7 Mix resin bottle well. Print in black resin with four 22x50mm chambers or eight 22x22mm chambers. Print chambers and lids directly on the printer platform without any rafts or support.

8 Printing should be done with light cover in place.

9 Printer and open resin should preferably be located within a chemical fume hood.

10 All handling of resin should be done with nitrile gloves, safety eyewear and a NIOSH organic carbon filter mask.

11 Clean up any spilled resin with 99% IPA and paper towels, or UV irradiate the area and chip off hardened resin for disposal. MSLA Post Processing

12 After printing, release 3D prints from the platform with a plastic scraper and wash for 15 minutes in fresh 99% isopropyl alcohol (IPA) in a Anycubic Wash & Cure Plus Machine (Anycubic, Shenzhen, China). Alternatively, prints can be washed in fresh IPA in a sealed polypropylene plastic container with vigorous shaking for 15 minutes. Thorough washing is essential to remove toxic free monomers and IPA should not be reused.

13 Prints are then cured under 405nm light in the same machine, for 30 minutes on the built-in rotating turntable. Alternatively, a 50W 385nm flood lamp (80mW/cm2) (WOWTOU, China) can be used: place face down on a 3D-printed box for 30 minutes, flipping once to avoid shadows. Any UV light source such as a UV light box can be used as an alternative, but move and flip the prints to avoid shadows.

14 Save used IPA for cleanup, but do not reuse the IPA for print washes. Chamber assembly to Coverslips

15 Use a 30mm diameter, 15cm wide craft roller to spread silicone RTV glue (SS-433T, Silicone Solutions, OH, USA) onto a 25X25 sheet of phenolic resin or glass plate.

16 Once the glue is spread evenly, use the roller to evenly transfer a thin layer of glue to the bottom surface of the inverted chamber.

17 With gloves on, gently press a 22x22mm #1.5 glass coverslip (VWR, USA) onto the glue face with a 3D printed tamper block (Supplemental Video 1). Visually inspect the glue surface to ensure complete contact and no voids.

18 Allow the glue to fully cure for at least 16hrs in the humid environment of a tissue culture incubator, to accelerate the curing time for RTV silicone. Alternatively, 72 hours without humidity incubation. Chamber Sterilization

19 In a HEPA tissue culture hood, bathe both chambers and lids 70% IPA for 10 minutes in a polypropylene tub, followed by filtered sterile water wash.

20 Remove from the bath and allow chambers and lids to dry under UV hood light and HEPA air flow on a sterile surface.

21 Place chamber and lid in a 5x10cm clear polypropylene bag under aseptic handling conditions with small sterile forceps.

22 UV irradiate the bagged chambers and lids prior to storage. Complete UV sterilization using either a 30mW/Cm2 365nm ELC-500 UV (ELC-500, Electro-Lite, USA) chamber on a rotating

platform for at least 10 minutes per side, or the flood lamp curing chamber previously described for at least 30 minutes per side (see Fig. 3).

23 Repeat UV irradiation of the bagged chambers and lids prior to use. Do not attempt heat sterilization by autoclaving.

Spotlight video

24

https://www.youtube.com/embed/xHwbZsjLqNs

Version 2.0 Update, August 2024

25 We have encountered a problem with MSLA resins in that there is an increasing amount of toxic UV crosslinking monomers appearing in consumer resins. This is from consumer demand for faster curing times. We attempted to switch to biomedical ISO certified engineering grade resins with time consuming post processing steps, but despite certifications, we could not find a resin that did not yield toxicity on cell culture.

Thus, we have switched to a filament deposition modeling, FDM, printing protocol using thermal polyurethane, or TPU, at 95A Shore hardness. This is for a few reasons: first, this plastic has excellent layer adhesion and strength, such that two walls of filament will yield water tight prints; second, the plastic is extremely thermostable, allowing autoclave or oven sterilization, and allows the use of a UV and thermal curing adhesive; third, without any warping, we can now print multiwell chambers on 22x50 mm #1.5 glass coverslips.

We have now switched away from RTV silicone to a UV and heat curing adhesive, Norland Optical Adhesive NOA 86H (Norland Optical Adhesives, Jamesburg, NJ, USA). This adhesive can offer a very flat adhesive layer, optically clear and strong with 15 minutes of exposure to a 385nm LED flood lamp. The adhesive is then cured for 3 hours at 80C or 1 hour at 120C to render an inert plastic adhesive interface, in which even primary human fibroblasts can grow without any toxic inhibition, and with less toxicity than commercial clear bottom chambered slides. This is more reliable, better quality than previous use of RTV silicone. We tested other NOA formulations but found them all to be toxic.

We have uploaded 3D print files for new 2, 4, and 8 wheel chamber designs in 22x50 format, as well as new holders. With TPU, these 22x50 chambers do not warp under heat or humidity conditions, unlike every other plastic filament attempted. Also, in this format, all wells are very flat as they all sit on one sheet of #1.5 glass (150um).

The new protocol is thus even more cost effective and requires fewer steps, with a better chamber quality similar to commercial products at US$12-18 each. We calculate chamber cost of 7 cents per TPU chamber, 0.5 cents per PLA lid, and 5 cents in adhesive, 10 cents per coverslip or under 23 cents each. This filament is best printed with any printer that uses a

direct drive print head, so that filament is not pushed through the Bowden tube. We used a smooth PEI steel magnetic bed so that the adhesive surface of the chambers are perfectly flat.

26 **Determining printing parameters**

1. Import models into any FDM slicer with standard settings used for 95A TPU. We recommend clear TPU for UV sterilization. Orient the prints with the coverslip face down on the print bed. This requires a modified chamber design and files have been updated.

27 2.Attempt pilot prints at the lowest temperature (~220C) to minimize stringing and no bed heat. Vary print temperature by 5C to minimize TPU oozing or stringing, but still retain layer adhesion for water tight prints.

28 3. Set printer toolhead movement to “combing” to avoid movement across the empty wells.This eliminates most stringing.

29 Set filament retraction to 3mm, but this will have to be empirically determined on each printer.

30 Once pilot prints have minimal stringing and good dimensional quality, scale up to 25-30 prints per bed (Figure 1a). This typically take 8 hours, or overnight. Coat the bed with PVA glue stick, as TPU can adhere too strongly to the smooth bed. This aids with print removal from the bed.

31 Print lids either clear or opaque in poly lactic acid, PLA.

Print Post processing

32 Use a small torch or heat gun to remove any wisps or strings from the print by a fast burnishing (Figure 1b and c).

Gluing

33 Use Norland Optical Adhesive formula 86H (NOA 86H). This adhesive is available in multiple viscosities, but the lowest viscosity ensures a flat surface by gravity flow.

34 Draw 1-2ml adhesive into a 5ml syringe with Luer lock, place on a 20 gauge needle, invert and displace any air. Wear gloves and avoid contact with the unpolymerized adhesive. Make sure there are no sources of UV light nearby (Figure 1d).

35 Dispense adhesive on the bottom surface (print bed surface) of the chamber, with minimal adhesive (Figure 1e).

36 Gently drop a 22x50mm #1.5 glass coverslip onto the adhesive and align (Figure 1f and g). Allow to settle by gravity only. A full seal should form.

37 Place the chamber under UV light at 375-385nm for 15 minutes (Figure 1h). The chamber is now fully adhered.

38 Heat chamber in lab oven for either 80C for 3 hours, or 121C for one hour. The temperature has to be above 80C to drive off the UV crosslinking toxic monomers. At 121C, the chambers are now sterile.

Sterilization

39 Use clear TPU for chambers and clear PLA for lids. Place chambers in a UV 260nm sterilizer (QKiss MS-208, China, about US$150) for 15 minutes, flip and expose another 15 minutes (Figure 1i).

40 Place chambers and lids in a small clear poly propylene zip bag and repeat previous step

41 Chamber can be re-sterilized again just before use, while in bags. Store at room temperature

notes

42

Avoid using the 260nm UV chamber to cure the adhesive, longer wavelength UV penetrates plastic deeper. The TPU chambers can be oven sterilized, UV sterilized, or 70% ethanol sterilized. However, lids cannot be heat sterilized. Clear PLA and TPU aids in sterilization by UV. If opaque TPU is used, move chambers in multiple orientations in the UV chamber to eliminate shadows.

The heat curing is essential to drive off volatile UV crosslinking monomers from NOA86H. This results in an inert plastic and cells can even grow on the plastic. All other UV adhesive formulas still have residual toxicity from monomers.

New FDM models for 22x50 format are available on the NIH 3D print exchange: **https://3d.nih.gov/** 3DPX-016293

With minimal training, a student can produce about 200 chambers in a week

Figure 1

43

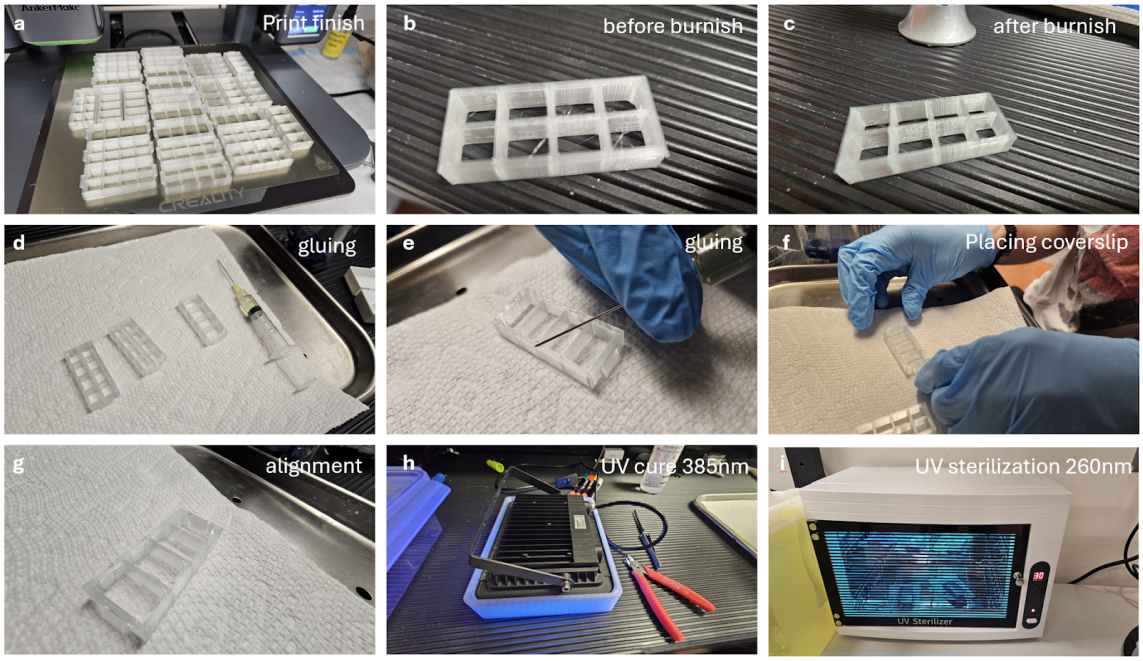

Figure 1. Mod3D 2.0 protocol by FDM printing with TPU. A, print completion. B, prints before flame burnishing to remove stringing, seen in C. D, Adhesive setup with NOA86H. E, gluing with a 20 gauge needle and placing coverslip by gravity only in F. G, alignment before 15 min. UV 385nm curing. After heating >80C for 3 hours, plates are allowed to cool, lids placed on and sterilized in a UV sterilizer at 260nm in clear bags with clear PLA lids.