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# FLYDE Plate: Open source anesthesia labware for *Drosophila* fly-pushing!

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**Abstract**:
One of the most important pieces of equipment used in labs in culturing populations of fruit flies (*Drosophila* sp.) is that of the "$CO_2$ gas plate", which is used to anesthesize individuals during "fly-pushing". This piece of equipment consists of a box with a porous top into which carbon-dioxide is pumped. Flies placed on its surface are left immobilized, permitting the sorting, categorizing and/or counting of flies during population culturing and experimental assays. Unfortunately, commercially available gas plates are typically expensive. Here, we describe a new design for a gas plate that can be easily produced using a 3D printer and a laser cutter, which we are making freely available to the fly community.

**Keywords**: Open source, 3D printing, *Drosophila*, laser cutter, lab equipment, open labware, fly-pushing, fly pad, fly plate, $CO_2$ anesthesia

## 1. Introduction:

The fruit fly, *Drosophila melanogaster*, has a long and illustrious history as a model organism in scientific research, and whose study has contributed to some of the greatest biological discoveries of all time (Weiner 1999, Brookes 2001). There are many, many, features that make *D. melanogaster* so amenable to a wide range of biological studies, not the least of which is the relative ease with which populations of flies can be cultured in the laboratory (Weiner 1999, Brookes 2001). Central to much of "fly-pushing" that comprises the culturing and maintenance of these populations, and their use in experiments, is the need to immobilize individuals so that they can be handled, examined, and sorted as needed (Markow and O'Grady 2005, Stocker and Gallant 2008). While temporary paralysis was can be achieved using dimethyl ether or triethylamine (the active agent in FlyNap®), these chemicals are quite volatile and/or a potentially toxic to humans by when inhaled or through contact with the skin (Artiss and Hughes 2007). Today fly immobilization is typically done using the (safer) methods of chilling using ice-baths (*e.g.* Perry 2018) or using carbon dioxide ($CO_2$) (Barron 2000). In the latter case, immobilization of flies is achieved by continuously piping this gas into the bottom a specialized staging apparatus: a box with a porous top. The $CO_2$ diffuses out of the box across the porous, keeping the flies on the surface anesthetized until they are removed. While these $CO_2$ staging apparatuses (which we colloquially shall hereafter refer to as "fly plates") are available commercially, they typically retail at well over $100.00 USD apiece. This price-point puts them outside the budget of many (actual and potential) fly researchers, and serves as a potential financial barrier.

The challenge posed by expensive fly-plates has been long recognized by researchers, some of whom have developed cheaper alternatives, which we shall briefly discuss. Melo Sene and Manfrin (2001) published plans for constructing an open-topped box apparatus out of acrylic sheeting. While this design appears robust to the physical stresses associated with fly-pushing, the presence of lateral walls completely surrounding the fly-pushing surface potentially makes it hard to remove flies without loss. Artiss and Hughes (2007) developed a economical fly-plate by modifying a micropipette tip container. While very ingenious, the use of a fabric top to the plate potentially makes keeping the apparatus clean and uncontaminated a challenge, and the lack of a "back-splash" wall increases the chance that some flies may accidentally fall off the back/sides of the apparatus. Furthermore in both of these aforementioned designs, the construction process is also fairly complicated, involving many precise cutting, drilling and gluing steps.

Some of the complications involved with manually constructing fly-plates is potentially avoided by using "3D-printing" (*aka* additive manufacturing) devices, in which virtual models are designed using Computer Assisted Design (CAD) software, and materials are combined, under precise computer-control, to manufacture desirable objects. There is an increasing use of personal-fabrication equipment and design-sharing – the open source movement – among scientists (*see* Pearce 2013, Baden 2015). To the best of our knowledge, only one open source plan for a 3D printed fly-plate exists (Cruz 2017). In this model, the entire apparatus is printed as single piece, with the bottom, and walls of the fly-plate printed at 100% infill with the interior and top of the box printed with to 85%.

While this design has many desirable features (including a back-splash), the flat upper printed surface produced by 3D printers can often be rough, potentially catching (and damaging flies), and is harder to keep clean.

Here, we describe the process by which we have designed, produced and tested a new $CO_2$ staging apparatus ("FLYDE plate"), as an open-source labware for the benefit of fly-pushers everywhere.

## 2. Materials and methods

In designing our fly-plate, our primary objective was to create a high quality, low-cost, apparatus that could with withstand the rigors of regular and prolonged lab use. Our fly-plate design consists of two pieces: a lower box, built using an additive 3D printing process (Figures 1 & 2) and an upper plate that was cut using a laser from a piece of acrylic sheet (Figure 3). The two pieces slide together (Figure 4), with gas introduced via a ¼" (outer diameter) vinyl tube that is inserted through a hole that is part of the lower box's design.

The lower box was produced on a Cubicon Style 3D printer (HyVision Systems) using a 0.4mm extruder from polylactic acid (PLA) filament. Production of the box used approximately 80g of PLA, which retails at approximately $45.00CAD/kg.

The upper plate was cut using a Transon TS6090 $CO_2$ Laser Machine (Jinan Transon CNC Equipment Co., Ltd.) from a 10.5 x 12.5 cm sheet of 3mm thick clear cast acrylic sheeting. The cost of a 4 x 8 foot sheet of this sheeting retails at approximately $45.00CAD. When cutting, the laser was set at 35% power. The laser cutter was used to perforate the surface of the acrylic sheet with 14,000 holes, each 0.25mm in diameter.

## 3. Results/Discussion

The completed FLYDE plate measures 3.2 cm high, 13.2 cm wide and 11.0 cm deep and has an active fly-pushing surface of 131.25 $cm^2$ that sits 2cm off the bench surface. The raised ("back-splash") walls around the back and side of the plate reduce the chances of flies being lost accidentally from the working surface. The perforated surface of the upper plate extends to the front edge of the apparatus, allowing for the seamless transfer of anesthetized flies from the surface of the FLYDE plate into a waiting vial.

Tests of the FLYDE plate have proven successful at providing a fairly even diffusion of $CO_2$ across the upper surface of the acrylic plate. This means that

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there were no zones where some flies were able to recover from the $CO_2$ while others remained immobilized. (Figure 5, Supplementary Video: https://youtu.be/UpzqK-EdZh4). A slower rate of gas diffusion can be produced by placing a thin sheet of tissue paper (*e.g.* Kimwipes Delicate Task Wipers, Mfr. No. 34155, Kimberly-Clark Inc.) in between the upper plate and the lower box. When assembled, the FLYDE plate was capable of withstanding the regular wear-and-tear associated with fly-pushing, and could be easily cleaned after use.

Our design is not without some potential limitations. First, it does require access to both a 3D printer and a laser-cutter, which may pose a logistical challenge to potential users. Secondly, the sheer number of holes in the top plate (14,000, each 0.25mm in diameter) means that its production can be time-consuming (and may pose a challenge if the laser-cutter does not have sufficient cooling systems). Thirdly, as the upper surface is composed of acrylic, it may conceivably be prone to static cling under some circumstances, which can adversely affect the efficiency of fly-pushing. While we have not encountered this problem, the use of an in-line bubbler may mediate this issue.

Ultimately it is our hope that the FLYDE plate will be adopted as a cheaper alternative to commercially produced fly plates, allowing more people to experience the wonders of pushing flies.

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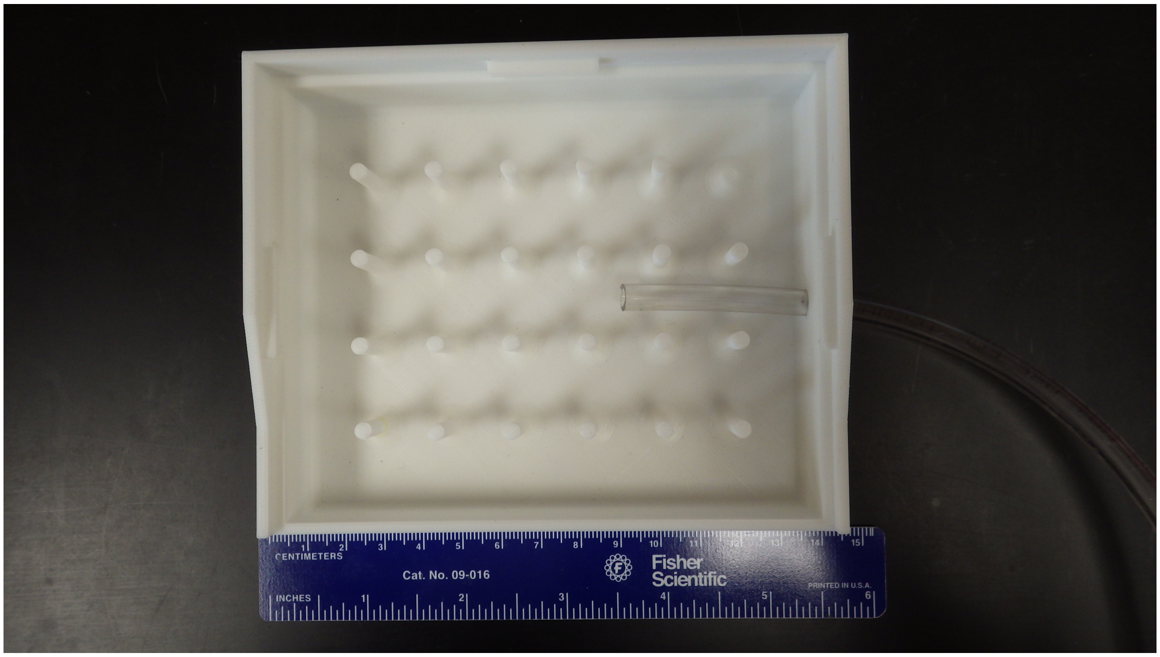

Figure 1: Side view of the 3D printed lower box portion of the FLYDE plate. Gas is introduced to the chamber via the inserted tube.

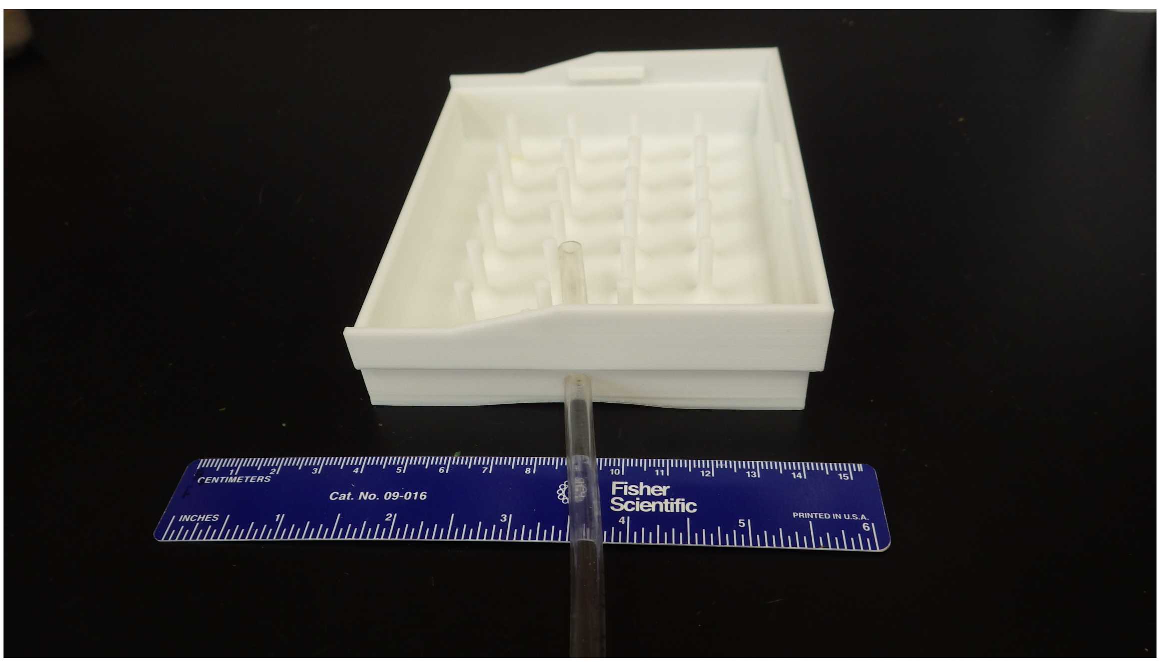

Figure 2. Side view of the 3D printed lower box portion of the FLYDE plate.

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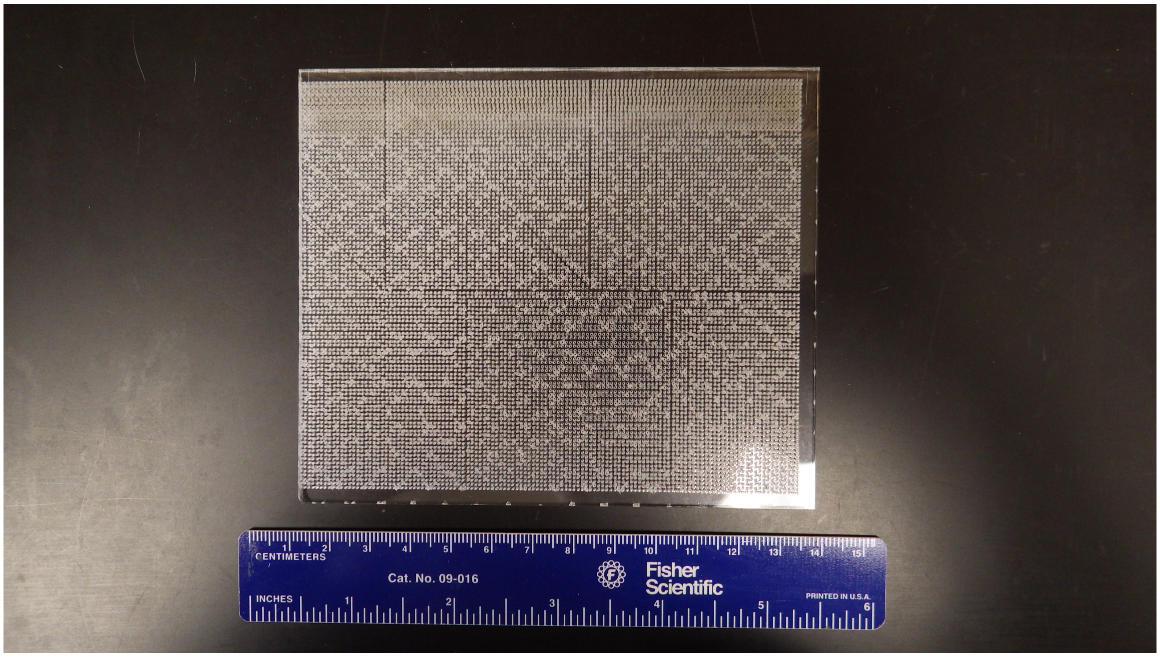


Figure 3. Photo of the laser-cut acrylic sheet that comprises the upper plate of the FLYDE plate.

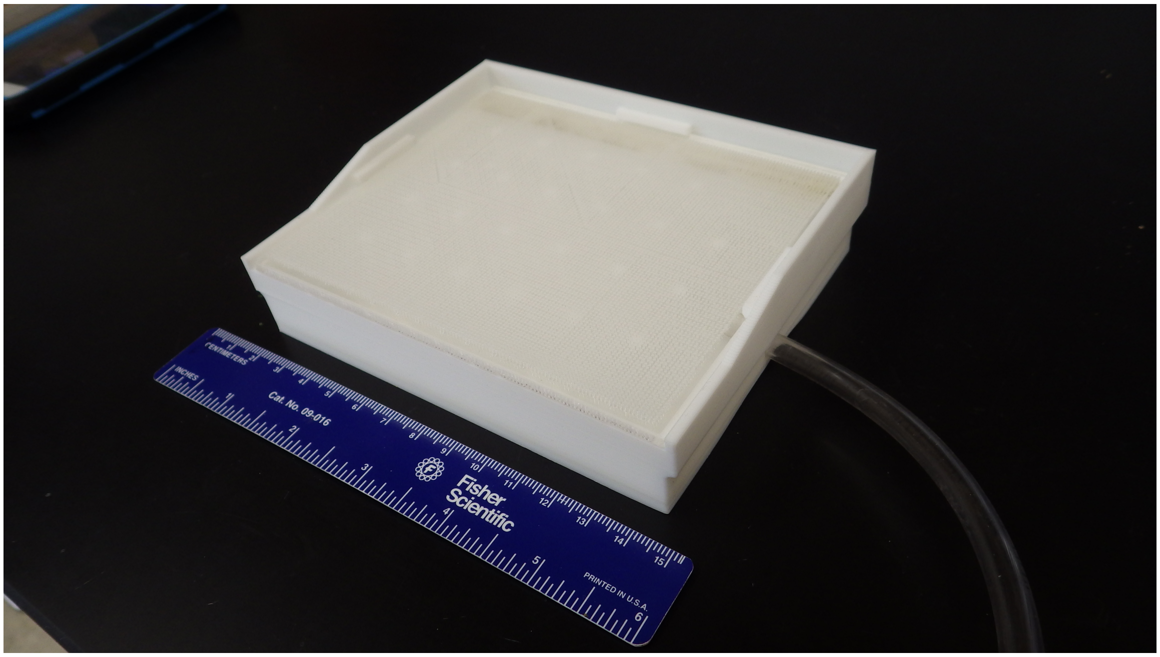


Figure 4: Photo of the assembled FLYDE plate. $CO_2$ is introduced to the bottom of the apparatus via the tube (on right), and dissipates through the upper plate.

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Figure 5: Photo of the assembled FLYDE plate. $CO_2$ is introduced to the bottom of the apparatus via the tube (on right), and dissipates through the upper plate. The tight grouping of the holes ensures that there is an ~even diffusion of gas across the surface, ensuring a consistent arena for manipulating anesthetized flies.

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**References**:


Artiss, T. and Hughes, B., 2007. Taking the headaches out of anesthetizing *Drosophila*: A cheap & easy method of constructing carbon dioxide staging. *The American Biology Teacher*, 69(8) 77-80.

Baden T, Chagas A.M., Gage G, Marzullo T, Prieto-Godino LL and Euler T., 2015. Open Labware: 3-D printing your own lab equipment. *PLoS Biology*, 13(3) e1002086.

Barron, A.B., 2000. Anaesthetising *Drosophila* for behavioural studies. *Journal of Insect Physiology*, 46(4), 439-442.

Brookes, M., 2001. Fly: the unsung hero of twentieth-century science. New York: Ecco.

Cruz, J. 2017. Fly pad. Model ID 3DPX-007884. https://3dprint.nih.gov/discover/3DPX-007884

Markow, T.A. and O'Grady, P., 2005. *Drosophila: a guide to species identification and use*. Elsevier.

Melo Sene, F., and Manfrin M.H., 2001. A $CO_2$ chamber for anesthetizing *Drosophila*. Drosophila Information Service. 84, 186-187.

Pearce J.M., 2013. *Open-source lab: how to build your own hardware and reduce research costs*. Elsevier.

Perry, J., 2018. “Glue filter paper down on top of a Petri dish packed with ice, works nicely [Tweet] https://twitter.com/jennyperry/status/1001119345404207106

Stocker, H., and Gallant, P., 2008. Getting started: an overview on raising and handling *Drosophila*. *Methods in Molecular Biology*. 420, 27-44.

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**Data Accessibility Statement**: All design files are uploaded to Thingverse.com (https://www.thingiverse.com/thing:3599321) and are available upon request from the authors

**Competing Interests Statement**: The authors report no competing interests.

**Author Contributions:** The project was conceived by TAFL, the design was a collaboration of JH and TAFL with drafting and model production performed by JH. TAFL and JH wrote the manuscript.

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