3D-Printable Micropipette

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Abstract

Open source development of lab equipment is being facilitated, in part, by growing availability of 3D printing which allows the production of simple lab tools. We developed 3D-printable parts that, along with a disposable syringe, form a micropipette. Once assembled the pipette requires no calibration or validation with a scale. Our printed micropipette is assessed in comparison to a commercial pipette demonstrating comparable performance in accuracy and precision and approaches ISO standard.

Introduction

The open source development model, initially applied to software, is thriving in the development of open source scientific equipment due in part to increasing access of 3D printing [1, 2]. Open design 3D-printable lab equipment is and attractive idea because, like open source software, it allows free access to technology that is otherwise inaccessible due to proprietary and/or financial barriers. Open design tools create opportunity for scientists and educational programs in remote or resource limited areas to participate with inexpensive and easy to make tools [1]. Open source development also enables the development of custom solutions to meet unique applications not met by commercial products that are shared freely and are user modifiable [3, 4]. Some advanced, noteworthy open-source scientific equipment include a PCR device [5], and a two-photon microscope [6] although even simple tools are impactful especially if the fabrication is simplified by 3D printing.

3D printing as a form of additive manufacturing has existed for decades although the recent availability of inexpensive desktop printers [**?**, 7] have made it feasible for consumers to design and print prototypes and even functional parts, and consumer goods [3]. Proliferation of free CAD software[OpenSCAD, Blender, SketchUp] and design sharing sites [**?**, 7–9] have also supported the growth and popularity of open designed parts and projects. Some simple and clever printable parts that have emerged are ones that give a new function to a ubiquitous existing device such as drill bit attachment designed to hold centrifuge tubes, turning the drill into a centrifuge [10]. Although this may make a rather crude centrifuge it may be and adequate method that only costs pennies compared to commercially available centrifuges. Other examples of open-design research tools that utilize 3D printed parts include optics equipment [11], microscopes [12, 13], syringe pumps [14], and reactionware [15] to name a few.

There are a few open design printable micropipettes including a popular one which uses parts scavenged from a retractable pen in addition to the printed parts [16]. An air displacement pipette uses a piston operating principal to draw liquid into the pipette.

In a typical commercial pipette the piston is made gas-tight with a gasketed plunger inside a smooth barrel. Consumer grade fused deposition modeling (FDM) printers are unable to form a smooth surface do to the formation of ridges that occur as each layer that is deposited. The ridges formed by FDM make it impractical to form a gas-tight seal between moving parts, even with a gasket. Printable open-design micropipettes get around this limitation by stretching a membrane over one end of a printed tube, which when pressed causes the displacement. The displacement membrane can be made from any elastic material such as a latex glove. After assembly this design requires the user to validate the volumes dispensed with a high precision scale. Without verification with a scale the volumes dispensed can only be estimated based on theoretical displacement intended by the design. There is no built-in feature such as a digital readout for the user to set the displacement to a desired volume. The pipette can be calibrated to a specific volume with a set screw but if a new volume is desired it has to be re-adjusted and validated with a scale.

We present a new open design 3D-printable micropipette that works by actuating a 1 mL syringe to a user adjustable set-point. This allows the user to set the pipette to a volume a priori by reading the graduations of the syringe barrel. The scale marks on the syringe can be used to adjust the dispensed volume accurately without calibrating with a scale. Our pipette also offers a simplified assembly requiring no glue, tape or permanent connections.

Materials and Methods

Design

Our printed pipette is designed to actuate a 1 mL or 3 mL syringe to a user set displacement. The core of the design is two printed parts, the body and the plunger which are able to be printed on a consumer grade FDM printer. A 1 mL BD syringe twists to lock in the body part and is held into place by the syringe flanges. The plunger part slides freely in the body part and actuates the syringe by pushing the thumb press. The pipette is spring loaded towards a set point which is adjustable by a set-screw. When the plunger is depressed the system locks when it reaches the latched position, where it is ready to draw in fluid. The plunger is held in place with a latching button design which is released with a lever drawing in fluid. The displacement is equal to the distance between the set position and the latched position. The pipette can also be pressed past the latched position to 'blow-out' the transfered fluid completely from the pipette tip. Additional materials required for assembly include a spring, a nut and bolt and washers. Attempts to make a printable luer lock adapter for tips was abandoned as the surface of printed parts is too rough to make an air tight seal with the luer or pipette tip. Instead a combination of a barbed luer adapter and elastic tubing was used to attach the pipette tips.

Our printed pipette mimics commercial pipettes's design, function, and user operation, making it intuitive to use. The button is pressed with the thumb until the latched position is reached. The the tip immersed in fluid and drawn in by actuating the lever with the palm or fingers. Conveniently this design can also reach to the bottom of a 15 mL conical tube allowing tasks such as aspirating supernatant fluid from a cell pellet where existing printed designs cannot.

Our pipette uses the air-displacement design, where a pocket of air is used to draw liquid into the pipette. As air is an expansible fluid, this pocket of air expands due to the weight of the liquid pulling on it. Due to this effect the graduations on the syringe are not accurate, as they are designed for measuring liquid within the syringe.

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Figure 1. CAD rendering of printable parts. Three parts are printed: the body, plunger shaft, and luer-lock adapter for pipette tips.

Figure 2. CAD renderings of printable parts. Two parts are printed: The plunger part (A) slides inside the body part (B).

when the liquid volume is pulling due to the force of gravity. This effect increases as the larger volumes are transfered. Due to the expandability of air the graduations on the syringe are not accurate. This effect is the greatest at larger volumes corresponding to a greater mass pulling on the air pocket. The pocket of air that is drawing in the water is stretched causing the inaccurate readings. We remedied this by creating a new scale to account for the expansion. The scale which is printed on a transparency sheet is pasted on the syringe as a guide for the set point.

Validation

The printed pipette was validated and compared to a commercial pipette. The pipette was tested to see if the set point would drift between during use for example small, incremental movements of the screw or other parts causing the pipetted volume to drift. To test the pipette the same volume was pipetted 50 times without moving or re-adjusting the set point. A small amount of paraffin wax was applied to the screw to prevent it from slipping during each actuation. The printed pipette's accuracy and precision was characterized and compared to a commercial pipette. The printed pipette and a series of commercial pipettes were tested at volumes of 10 μL, 20 μL, 30 μL, 50 μL, 200 μL, 300 μL, 500 μL and 1000 μL. The printed pipette was adjusted to the the target volume by eye from the syringe graduations, and the resulting volume was measured and repeated 5 times to account for variability. Measurements were taken from the commercial pipette in a similar way.

Results and Discussion

Our printed pipette had a comparable accuracy to a commercial pipette (Table 1). The commercial pipette has a digital readout that allows the user to adjust precisely to set values where the printed pipette is adjusted by eye according to graduations. Due to being unable to re-set the printed pipette back to the exact mechanical location it may be hard to compare directly with the commercial pipettes. This is certainly the cause of the lower accuracy and wider deviation although it's performance is impressive. Despite this additional variability the printed pipette's accuracy and precision approaches the ISO 8655 standard.

The Novice user data demonstrates that even someone inexperienced with pipetting and unfamiliar with our design is able to use out pipette accurately with out the use of a scale. The systematic error trended towards over estimating the displacement. This is likely due the function of adjusting the volume where it is easier to start at a lower displacement and increase it by unscrewing, or releasing compression of the spring. This explains the negative systematic error as the user avoids 'going over'.

This pipette improves on existing open design pipettes in several ways. It requires only two printed parts, a syringe, and some hardware. It is able to reach into a 15 mL

Figure 3. Photos of pipette. The pipette is composed of three printed parts, a 1 mL syringe and some additional hardware.

conical tube which is a routine requirement for cell culture. No permanent connections using tape or glue are required for assembly allowing worn out or broken part to be replaced easily. Assembly does not require a membrane that may wear or stretch and require tedious replacement. No validation or calibration is required. The pipette can be assembled and used to accurately pipette micro volumes without first validating with a scale.

The only major limitation of this design compared to the briopipette is the option for a pipette tip ejector, although incorporation of a similar design in our pipette would not leave room for it to reach into a 15 mL conical tube. Our pipette requires a syringe and some additional hardware where briopipette's additional parts can be obtained virtually anywhere. The briopipette also allows step-wise shifts in displacement allowing the user to quickly change to a relative volume

Table 1. ISO 8655 for 100-1000 uL comparing a commercial pipette with our printed pipette used with existing 3 mL syringe scale and an adjusted scale

		Mean	Systematic Error	% Sys. err.	Random Error	% Rand. err.
1000 uL	ISO 8655, 100-1000 uL	1000	8.00	0.80	3.00	0.30
	Commercial Pipette	1002.98	2.98	0.30	1.72	0.17
	Printed Pipette	949.29	-50.71	-5.07	0.60	0.06
	Printed Pipette Scale	1003.57	3.57	0.36	0.89	0.09
500 uL	ISO 8655, 100-1000 uL	500	8.00	1.60	3.00	0.60
	Commercial Pipette	503.67	3.67	0.73	0.49	0.10
	Printed Pipette	475.99	-24.01	-4.80	4.75	1.00
	Printed Pipette Scale	503.62	3.62	0.72	1.64	0.33
200 uL	ISO 8655, 100-1000 uL	200	8.00	4.00	3.00	1.50
	Commercial Pipette	204.61	4.61	2.30	0.15	0.07
	Printed Pipette	186.55	-13.45	-6.72	1.31	0.70
	Printed Pipette Scale	201.87	1.87	0.94	1.47	0.73
100 uL	ISO 8655, 100-1000 uL	100	8.00	8.00	3.00	3.00
	Commercial Pipette	104.29	4.29	4.29	1.65	1.58
	Printed Pipette	94.02	-5.98	-5.98	4.81	5.12
	Printed Pipette Scale	101.00	1.00	1.00	1.05	1.04

For more information, see **??**.

Supporting Information

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Table 2. ISO 8655 for 30-300 uL comparing a commercial pipette with our printed pipette used with existing 3 mL syringe scale and an adjusted scale

		Mean	Systematic Error	% Sys. err.	Random Error	% Rand. err.
300 uL	ISO 8655, 30-300 uL	300	4.00	1.33	1.50	0.50
	Commercial Pipette	301.19	1.19	0.40	0.53	0.18
	Printed Pipette	286.91	-13.09	-4.36	0.42	0.15
	Printed Pipette Scale	299.11	-0.89	-0.30	0.48	0.16
200 uL	ISO 8655, 30-300 uL	200	4	2	1.5	0.75
	Commercial Pipette	200.06	0.06	0.03	0.46	0.23
	Printed Pipette	193.40	-6.60	-3.30	2.86	1.48
	Printed Pipette Scale	200.57	0.57	0.28	0.86	0.43
50 uL	ISO 8655, 30-300 uL	50	4	8	1.5	3
	Commercial Pipette	49.02	-0.98	-1.96	0.10	0.20
	Printed Pipette	49.62	-0.38	-0.76	1.26	2.53
	Printed Pipette Scale	48.73	-1.27	-2.54	1.11	2.27
30 uL	ISO 8655, 30-300 uL	30	4	13.3	1.5	5
	Commercial Pipette	29.08	-0.92	-3.06	0.09	0.31
	Printed Pipette	29.22	-0.78	-2.59	0.31	1.07
	Printed Pipette Scale	27.78	-2.22	-7.41	1.37	4.93
20 uL*	ISO 8655, 30-300 uL	20	4	20	1.5	7.5
	Commercial Pipette	NA	NA	NA	NA	NA
	Printed Pipette	18.70	-1.30	-6.48	0.38	2.01
	Printed Pipette Scale	17.94	-2.06	-10.29	1.87	10.42
10 uL*	ISO 8655, 30-300 uL	10	4	40	1.5	15
	Commercial Pipette	NA	NA	NA	NA	NA
	Printed Pipette	11.95	1.95	19.52	0.73	6.08
	Printed Pipette Scale	7.64	-2.36	-23.64	0.38	4.92

* The 20 uL and 10 uL volumes are out of the range in this case but we wanted to demonstrate that the pipette is capable of even smaller volumes.

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