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# Simultaneous metering and dispensing of multiple reagents on passively controlled microdevice solely by finger pressing

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## ABSTRACT

In this work, we report a novel design of a passively-controlled, finger-driven microfluidic circuit for the metering and delivery (MaD) of liquid reagent. The proposed design modularized the fluidic circuit for single reagent's MaD so that it can be multiplexed conveniently for the MaD of arbitrary number of reagents solely by finger pressing. The microdevice has comparable accuracy with pipettes and we demonstrated its applicability in the preparation of biochemical assays. The proposed design of the modularized, structurally "stackable" fluidic circuit provides a reference in the designing of future single-pressure-source-driven, passively-controlled multi-liquid handling microfluidic platforms.

## 1. INTRODUCTION

The accurate manipulation of low volume (nanoliter to microliter) fluids is an evolving art in microfluidics. To control the direction of fluid flow and the flow rate, active microscale valves are routinely employed. While a variety of mechanical valves exist, the use of 'normally-open'[1] or 'normally-closed'[2] PDMS check valves is common, and these have led to large scale integrated microfluidic devices[3] and integrated 'all-in-one' microdevices[4,5]. However, the control of pneumatic check valves requires external pressure sources and often sophisticated electronic systems, which can have a relatively large footprint, limiting the use of microfluidic devices to the laboratory. To broaden the impact of microfluidic analytical devices, more portable and less expensive on-chip flow control strategies are needed; such advances will make the devices more accessible to a broader range of users, notably those focusing on the development and implementation of point-of-care diagnostics.

In contrast to actively-controlled valving, passive flow control is markedly less complicated and reduces the need for external hardware, as there is no need for the external pressure control of deformable membranes. Passive valving techniques such as abrupt capillary valves[6]–[9] and hydrophobic patch valves[10]–[12] have been shown to open when the inlet pressure exceeds a 'burst threshold' defined by the geometry and surface properties, and remain closed when the inlet pressure is beneath the threshold. However, many applications require valves that enable a broader range of activation pressures and/or extremely different activation pressures for each flow direction. Flap or membrane-based fluidic diodes are a promising example of such valves, and have been made from silicon, metals, parylene or SU-8 for various applications, especially in combination with

micropumps[13,14]. As a result of its elasticity and simplicity of mold-based fabrication processes, silicone has emerged as the leading material for fluidic diodes: for example, Santra et al. developed a fluidic diode-based micropump using silicone[15], while Adams et al. reported an all-PDMS fluidic diode with a hanging flap to prevent backflow[16]. Using the similar diode structure, Iwai et al. developed a finger-powered droplet generation and cell encapsulation system[17]. Leslie et al. used PDMS membrane-capped fluidic diodes to rectify the flow in the frequency-specific flow switching manner[18], while Mosadegh et al. reported automated oscillatory flow switching using a set of three-layer PDMS flap fluidic diodes[19].

A fundamental operation critical to the function of microfluidic devices is the precise metering and dispensing of desired volumes of multiple solutions in a desired ratio. This has been achieved by active manipulation of a series of check valves that require multiple, independently-controlled pressure sources, and/or complicated logic operations[20]–[24]. In contrast, by taking advantage of modified Mosadegh's three-layer fluidic diodes, Li et al. constructed a fluidic circuit that could be driven by finger pressure to achieve the metering and delivery (MaD) of liquid solutions[25]. However, the reported design suffers from some critical problems that prevent this concept of 'finger-driven-microdevice' being further extended to wider range of applications: (1) each reagent needs a specific finger pump to drive, which consumes significant space and requires redundant operations; (2) delivery of the reagent is performed by liquid-liquid discharging without an accurate end point, which leads to either insufficient or excess delivery of the metered solution and hence inaccurate result; (3) the controlling channels connecting from the finger pump to the fluidic diodes is not essential and unnecessarily complicates the design. All these problems make the extension of the design to the MaD of multiple (>2) reagent almost unachievable and severely limit the potential of the concept of the finger-driven microdevice within a few simple applications.

With the present work, we report a novel design of a modularized MaD fluidic circuit that: (1) allows for the parallel metering and all-in-one delivery of multiple reagents solely by one set of finger pressings on one single finger pump, without any redundant pumping structures or operations; (2) consists of modularized design that can be

conveniently multiplexed for the MaD of arbitrary number of reagents. In comparison with the previously reported microdevice[25], this finger-driven microfluidic device is more accurate as well as concise in both structures and operations, allowing for the handling of multi-reagents tasks. This is demonstrated by the dispensing of: (1) two reagents for colorimetric quantitation of protein solution, and (2) four reagents needed to create a mixture for the enzyme-mediated DNA amplification (PCR) in a manner comparable to conventional pipette-based reagent dispensing.

## 2. MATERIALS AND METHODS

Unless otherwise specified, all reagents were purchased from Sigma Aldrich.

### 2.1. Fabrication of glass-PDMS-glass (GPG) microfluidic device

In the fabrication of the fluidic diode, laser ablation was used to ablate down commercial PDMS film and form a thin membrane as the flap of the fluidic diode. Laser ablation-based micro-machining is gathering increasing attention because of its markedly faster prototyping speed, lower labor cost and higher level of automation[26]–[29]. The conditions for laser ablation of PDMS have been previously discussed by Liu et al.[29] and Forgarty et al[30].

In brief, features in PDMS layer was designed using CorelDraw 10.0 and then engraved by VersaLaser VLS 3.50 with 50W $CO_2$ laser source (Universal Laser System Inc.), into either a 290 μm (for diode and metering chamber) or 1.5mm (for finger pump) thick commercial PDMS film (Rogers Corp.). The diode flap was formed by partially ablating the PDMS film, and the metering chambers were created by complete cutting-through in the same film (Supplementary Information, Section 1). After brief sonication in ethanol to remove ash and residue, the PDMS layer was dried and the geometry of the feature was characterized by examination of their cross-sections under microscope.

The glass bottom layer of the microdevice was fabricated using conventional photolithography and HF etching. The mask was designed using AutoCAD and then manufactured in photographic film in high resolution (Fineline Imaging, CO). Borofloat glass plate (Telic, Valencia, CA) pre-coated with photoresist and chrome layers was etched by HF solution ($HF/HNO_3$:200/30 (v/v)) after UV exposure, development and removal of the exposed chromium layer. The photoresist and chromium remaining on the plate were

removed completely after the etching. The diode valve seats were protected using dilute photoresist using previously reported method[18], and then bonded with the PDMS layer using a conventional plasma bonding technique. Protected photoresist was removed by ethanol flushing after PDMS recovery. The whole procedure of fabrication is diagramed in Figure 1.

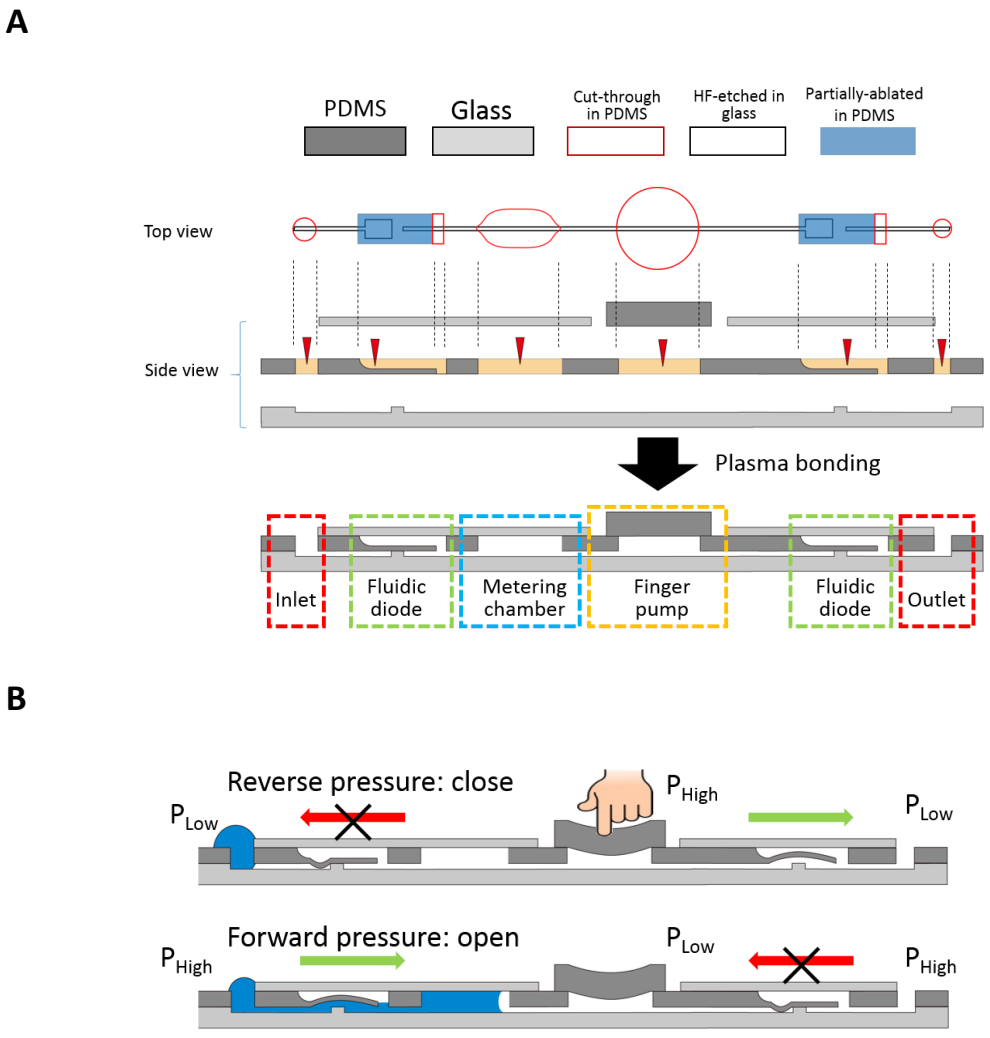


**Figure 1**. (A) Fabrication of a laser-ablated fluidic diode, together with other essential components (inlet, metering chamber and finger pump) fabricated in the same finger-driven microdevice (these structures are not in scale). The movable flap of the fluidic diode is laser-ablated in a single PDMS film and sandwiched by a valve seat layer (glass) and a top plate (glass). (B) Fluidic diode-enabled one-way liquid flow in a finger-driven fluidic circuit. Liquid flow is only permitted from the inlet through the diode under forward pressure.

### 2.2. Opening pressure determination

An empty 100 μL syringe (Hamilton) was connected with a flow chamber that has a diode in the inlet channel (Supplementary Information: Fig. S2). Initially the system (syringe, fittings, tubings, channels and flow chambers) are filled with air at room temperature. We used a camera to record the experiments the infusion of liquid into the flow chamber. We used a syringe pump (neMESYS Syringe Pump, Cetoni) to start pulling the syringe at a fixed flow rate, so that the volume of air in the system as well as the inner pressure of the system can be calculated at a given time point(Supplementary Information, Section 2). The opening pressure (defined in 3.2) was experimentally determined at the time point when solution started to infuse.

### 2.3. Design of MaD device and assembly

The basic structure and the stepwise operations of a finger-driven MaD circuit are shown in Figure 2. In the first stage, air in the finger pump is expelled from the device by finger pressure that deforms the PDMS plate of the pump. In the second stage, after releasing of the finger, the deformed PDMS will relax to its initial state and draw solution into the metering chamber. After the complete filling of the metering chamber the excess liquid enters the pump but does not completely fill it, because the replaced air from the metering chamber is drawn into the pump and stored together with the remaining air from the preceding stage. In the third stage, the finger pump is depressed again, and the remaining air stored in the pump will replace the solution in the metering chamber until all metered solution is completely discharged into the outlet, with the excess liquid still in the finger pump. This cycle of operation can function repetitively.

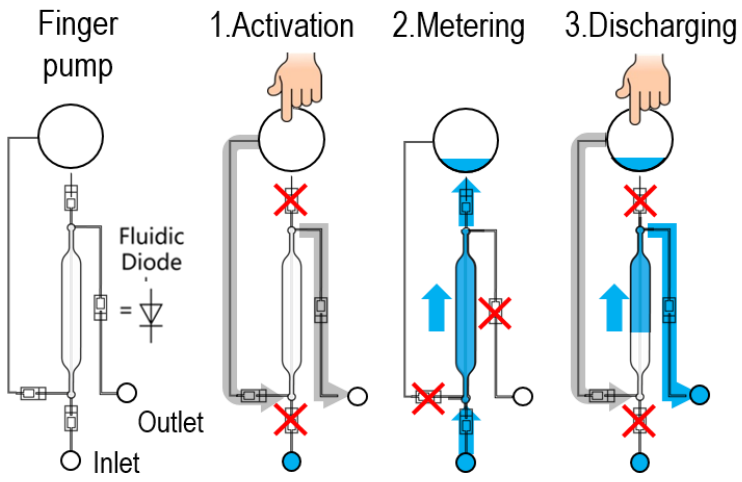


**Figure 2**. The operation and procedural details of the finger-driven MaD. The MaD operation is accomplished using two sequential finger compressions in three steps.

## 2.4. Determination of volume metered and delivered

All pipettes used were calibrated before the experiments of 2.4-2.7. Blue dye solution was prepared using erioglaucine at concentration of 6 mM in 1X Tris-EDTA buffer (pH 7.5) (Sigma Aldrich). Approximately 20 µL of blue dye solution was pipetted into the inlet reservoir and forced into a series of metering chambers varying in volumes from 0.15 µL to 9 µL. The solution delivered into the outlet reservoir was pipetted out and be diluted to 500 µL using 1X Tris-EDTA buffer (pH 7.5). Diluted solutions were then analyzed by UV-Vis spectrometer (Shimadzu) at 427 nm to determine the delivered volumes.

## 2.5. Determination of mixing ratio of multiple solutions delivery

Erioglaucine, tartrazine and alura red solutions were prepared at concentration of 6mM, 15mM and 30mM, respectively, in 1X Tris-EDTA buffer (pH7.5). Dye solutions as well as 1X TE buffer (pH7.5) were metered in specific volumes and delivered to mix. 5 µL of delivered mixture of four solutions was pipetted out and diluted to 500 µL by 1X TE buffer (pH7.5). Diluted solutions were then analyzed by UV-Vis spectroscopy (Shimadzu) from 300nm to 700 nm to determine the overall absorbance spectrum, and then the fraction

of each solution was calculated by their extinction coefficient matrix (Supplementary Information, Section 3).

### 2.6. Colorimetric test of protein solution

A commercial protein quantitation kit, Coomassie Brilliant Blue G(CBBG) solution, was used to demonstrate the delivery of two reagents for a rapid quantitation of total protein concentration. Equal amount of CBBG solution and Bovine serum albumin (BSA) solutions at a varying concentrations (12.5, 25, 50, 100, 200 μg/mL) were metered, delivered and mixed either using pipette or finger driven microdevice. Mixed solutions were placed in room temperature for 1min for complete chromogenesis and then pipetted into a 290 μm high glass-PDMS-glass chamber and get scanned (Epson Perfection V100 PHOTO, Epson). Scanned images were analyzed using imageJ and the saturation values were used to quantitate the concentration of protein (Supplementary Information: Section 4). To avoid adsorption of the CBBG on the surface of the glass, before each performance of MaD, all metering chambers were pre-loaded with CBBG then aspirated to get air dried.

### 2.7. Polymerase chain reaction preparation

A commercial PCR kit (AmpFLSTR® Identifiler® PCR Amplification Kit, Life Technology) for amplification of short tandem repeats (tetra and penta nucleotide) at 16 different loci in the human genome was used to assess the biochemical compatibility and performance of the finger-driven microdevice. Briefly, 2 μL of *Taq* Gold polymerase, 15 μL of standard DNA, 15 μL reaction mix and 15 μL of DNAse-free distilled $H_2O$ were added to their respective reservoirs prior to the operation. A total volume of 25 μL of the mixture was dispensed after the three cycles with the finger-driven MaD of each solution in their specific needed volume (Supplementary Information, Table S1). Prepared PCR mix was run in a thermal cycler (Bio-Rad) and the amplicons were analyzed by capillary electrophoresis (ABI 300).

## 3. RESULTS AND DISCUSSION

### 3.1. Opening pressure of the fluidic diode

A functioning fluidic diode should be able to open under forward pressure (Figure 1B). We define the pressure required to open a fluidic diode as the 'opening pressure' (OP). Investigation of the OP is critical in understanding whether a human finger can provide adequate pressure to open a fluidic diode and drive flow. We experimentally determined the OP for different thickness of the flap of a fluidic diode and the trend of increasing OP with increasing flap thickness (Fig. 3A). The increased opening pressure reflects the increased energy required to deform the flap and delaminate the PDMS from the glass valve seat.

As shown in Figure 3A, for the flap thickness used in the finger-driven MaD circuit, which is 60 μm in this case, the threshold OP is ~9 kPa. And a ~10% fabrication variation (Supplementary Information, Section 1) of the thickness around 60 μm gives an approximately ±1 kPa variation of the opening pressure, which is acceptable for finger actuation discussed below. The finger pressure, and the subsequent PDMS relaxation, provide the driving pressures for the discharging and metering flow, respectively. In order to open the diodes to initiate flow, those two pressures should be higher than the OP. The force that can be applied by a human finger is gender-dependent and ranges from 35-60 Newtons (N) for a male and 21-36 N for a female[31]. Therefore, the force applied by the weakest human finger on our finger pump (area = 50 $mm^2$) generates a pressure of ~420kPa, which is two orders of magnitude higher than the device's OP.

### 3.2. Resistance of the fluidic diode in the open state

The resistance of the fluidic diode is extracted from the flow profile after the fluidic diode is opened (Supplementary Information, Section 2). The relationship between fluidic resistance and pressure difference shows a trend of decreasing resistance with increasing ΔP (Fig.3B). The resistance reaches a stable level that represents the baseline resistance of the upstream and downstream connecting channels and a fully-opened diode. As the pressure decreases, an opened diode will approach the closed state, and the fluidic diode enters a bi-state transition stage wherein it is sensitive enough to be switched by small perturbation between the open and closed states, generating unpredictable discrete flow. This is reflected in the markedly increased resistance and associated error when the ΔP is

lower than 4 kPa, the point that we define as the threshold pressure for an already opened fluidic diode to remain open.

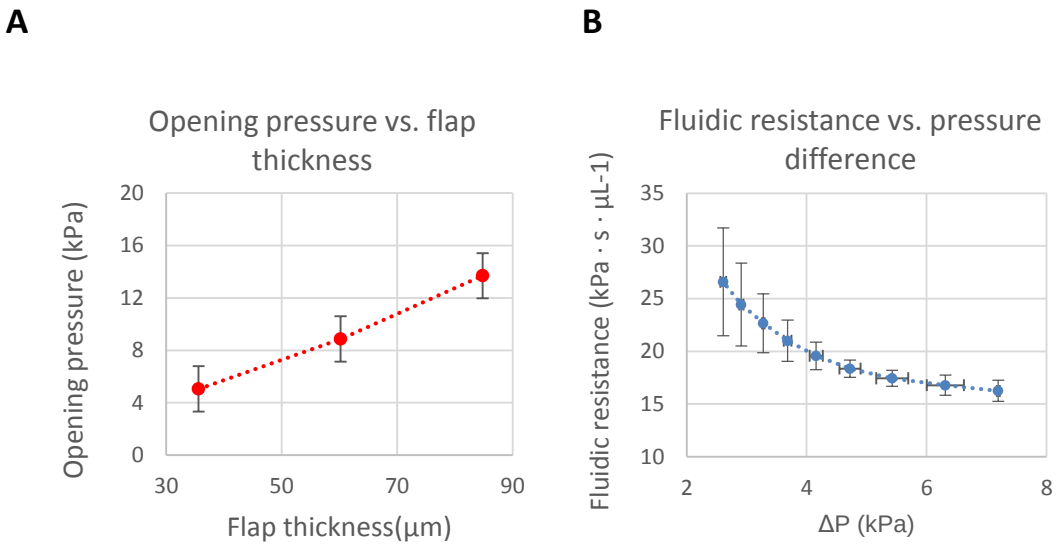


**Figure 3**. Characteristic of the fluidic diode. (A) Open pressure of fluidic diode at forward direction versus flap thickness. (n=3) (B) Relationship between fluidic resistance and pressure difference across the fluidic diode. (n=4)

### 3.3. A fluidic diode-based finger-driven MaD process

The fluidic diodes were used in a finger-driven microfluidic circuit, and the expected MaD process was successfully demonstrated (Fig. 4). In the metering stage, no introduction of air was observed through the closed diode and, in the delivery stage when finger pressure is applied to the pump, no air was pushed out back to the inlet. The entire process associated with a single MaD cycle was complete in 20 sec, and multiple cycles (n=3) could be done consecutively.

The most distinguishable feature of our design from the previous design[25], is that we successfully separated the metering and the delivery process in a single finger pump's operation as shown in Figure 2. Unlike Li et al.'s design where for even one reagent at least two pumps are required in the metering and the delivery processes, we assign the metering and the delivery processes to the drawing (Fig. 2, step 2)and pushing

(Fig. 2, step 3) of a single finger pump, respectively so that no redundant pumps and operations are required. In addition, the delivery of the metered solution is driven by the discharging of the air in the pump. With a clear gas-liquid interface indicating the end point of the delivery, all the metered volume can be delivered much more accurately than previous design (liquid-liquid discharge). All this novel features enable the metering and the delivery processes to be conveniently multiplexed as will be described in details in section 3.5.

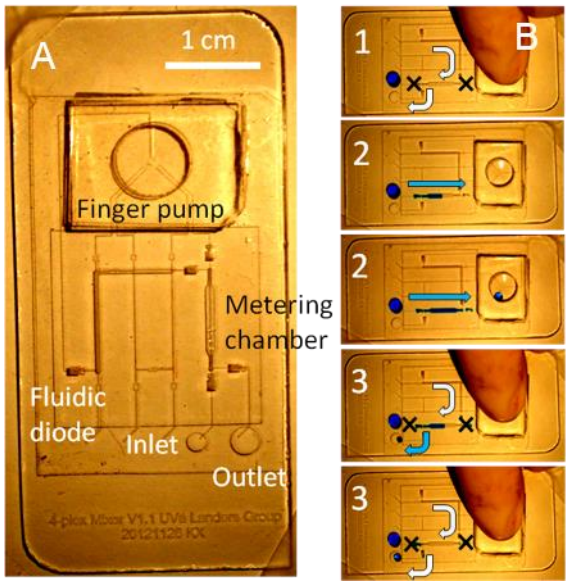


**Figure 4**. The MaD process in a finger-driven microfluidic chip. (A) The chip and labeling of the fluidic components. (B) stepwise snap shots of the MaD process.

### 3.4. Precision and accuracy of the MaD process

During the metering process, the internal pressure of the system is lower than ambient pressure and, thus, the chamber floor and ceiling of the metering chamber experiences significant deformation, which can contribute to errors in the metered volume. To minimize such error, we used a glass-PDMS-glass structure to provide a much more rigid chamber floor and ceiling than that provided by an all-PDMS system. The precision of metered volumes (Fig. 5, $R^2$=0.99995, 3% standard error) was markedly better than previously reported in an all-PDMS finger-driven microdevice[25].

The slope of the correlation in Figure 5 shows that the volume of solution discharged in the outlet after MaD is ~3.9% less than designed values. However, we did not observe any residual solution in the metering chamber following discharge. We attribute this deviation to the roughness of the laser-cut PDMS side-wall in the metering chamber[29]. It is possible that microscale 'hydrophobic pockets' may form at those concave sites during the infusion of aqueous solution, and a very small fraction of air is trapped, decreasing the amount of solution that is metered. The deviation is comparable with the average error of a 20 µL micropipette.

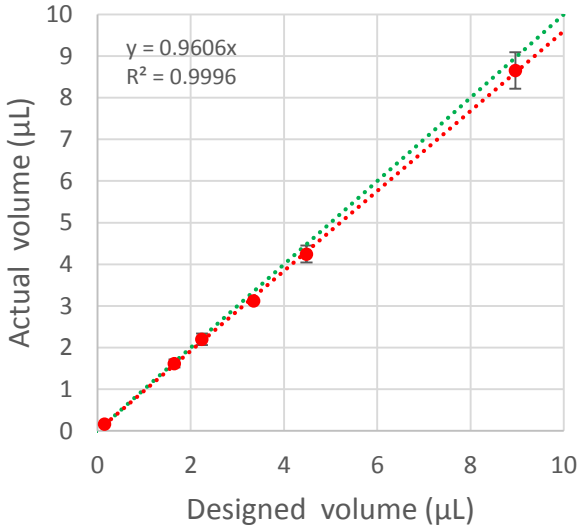


**Figure 5**. The correlation between the actual MaDed volumes and designed volumes ranging from 160 nL to 9 µL (n=3).

### 3.5. MaD of multiple reagents

In the previously reported finger-driven chip[25], the delivery of the proceeding solution and the metering of the subsequent solution are not completely independent (the metering of the late-coming solution requires the delivery of the already-metered, first-coming solution). This makes the procedure of the MaD of different reagents running in a sequential manner, which suffers from the difficulties of parallelization.

By re-design and modularize the single-reagent MaD fluidic circuit into an expandable unit, the multiplexing of the MaD process becomes rather convenient (Figure

6A). The fluidic circuit for the MaD of arbitrary number of reagents can be easily designed by simple replication of the modularized MaD unit (except each unit's specific metering chamber with needed volume). When dispensing multiple reagents (Figure 6B), the metering processes of all the reagents can be performed in parallel without alternating the delivery process. The parallel metering processes only requires a single finger pump with only one time of actuation, and the overall delivery of all the reagents is still driven by the same finger pump with only one time of pressing. No redundant finger operation is needed.

To demonstrate this, we fabricated a fluidic circuit with four MaD units each having a corresponding inlet and metering chamber with a desired volume, and connecting to a single common outlet in series (Fig. 6A). In a single MaD unit, only a type I diode (Mosadegh et al. [19]) is needed to prevent back-flow. In the dispensing of multiple reagents, however, type II diodes (Leslie et al. [18]) are also required. The function of type II fluidic diode is to prevent cross-flow from either side between two adjacent MaD units during metering. To test the ratio of components in the final delivered mixture (Supplementary video 1), four solutions were used, three containing dyes [allura red (red), erioglaucine (blue), tartrazine (yellow)] and a fourth colorless buffer (1X TE, pH 7.5), The red, blue, yellow and colorless solutions were drawn into circuits having volumes of 0.15, 1.65, 3.2 and 3.3 μL, respectively. The significance of these volumes will be expalined in section 3.6. The requisite initial concentration of each dye solution was calculated based on their volume ratio during the preparation so that they have minimum overlap in spectrum and all dye solution's absorbance peaks could be well resolved (Fig. 7A).

After all of the metered solutions were delivered to the outlet reservoir, a fraction (5 μL out of 8.3 μL) of the mixture was pipetted out for spectral quantitation and volumetric calculation (Supplementary Information, Section 3). Figure 7B&C shows that the overall spectrum and the ratio of all four solutions has less than 6% deviation from standard reference.

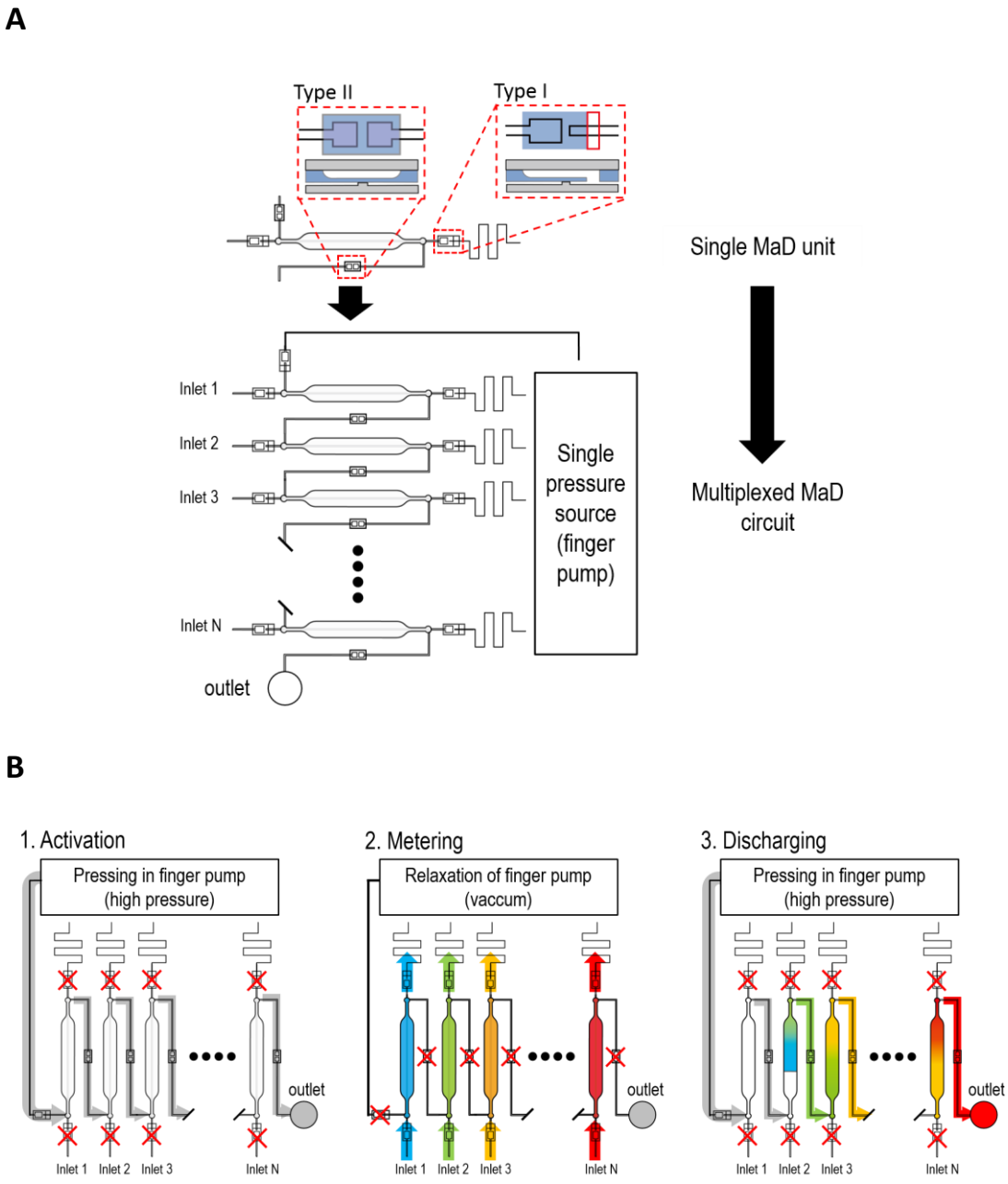


**Figure 6**. MaD of mutiple reagents on a finger driven microdevice. (A**)** The structure of a multiplexed MaD circuit by serial connection of multiple single MaD units. Two types of diodes are shown in both top view and cross-sectional view. (B) A step-wise schematic showing a fluidic circuit with N stackable MaD units for the MaD of N different reagents.

The designing of the multiplexed MaD fluidic circuits also presents a perfect elaboration of an idea that has long been highly valued in the designing of microfluidic devices: being structurally 'stackable'. The single MaD unit can be easily "stacked"

together and scale up to handle the arbitrarily multiplexed liquid handling tasks without adding other complexities, which releases the potentials of larger scales of integration for more sophisticated tasks in the future.

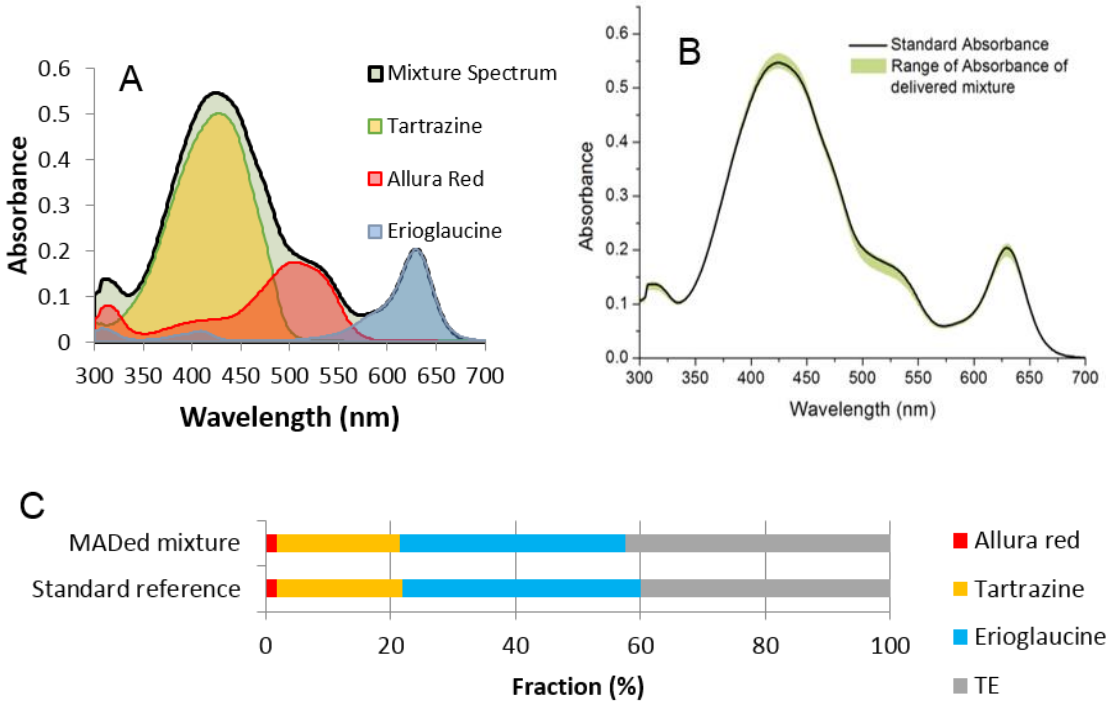


**Figure 7**. The accuracy of finger-driven multi-reagent MaD process. (A) The spectra of the reference mixture containing three different dye solutions at the desired ratio. (B) The comparison between the spectrum of reference mixture and the on-chip metered and delivered mixture with the range shown in green (n=3); (C) Comparison between the ratio of components in the MaD-ed mixture and standard reference

### 3.6. Colorimetric quantitation of protein on finger-driven microdevice

A potential application of the finger driven microdevices is to provide platforms for point-of-care diagnostics where sensitive, simple and rapid tests are highly desired. Total protein concentration in body fluid is a significant indicator of health conditions, and convenient monitoring of protein level is of great significance in the prevention and early treatment of malignent diseases such as uremia. We transplanted a CBBG-based rapid

colorimetric test of protein onto the finger driven microdevice and used a home scanner to detect the signal intensity (Supplementary Information, section 4). The saturation values of the scanned images of CBBG-protein solution in the detection chamber is linearly correlated with the logarithm of protein concentration in μg/mL (Supplementary Information, Fig. S3B). A comparison between finger-driven microdevice and pipette in quantitation is shown to have less than 3% difference (Fig. 8). The range of detection covers the normal range of the concentration of total protein (20-150 μg/mL) in urine[32]

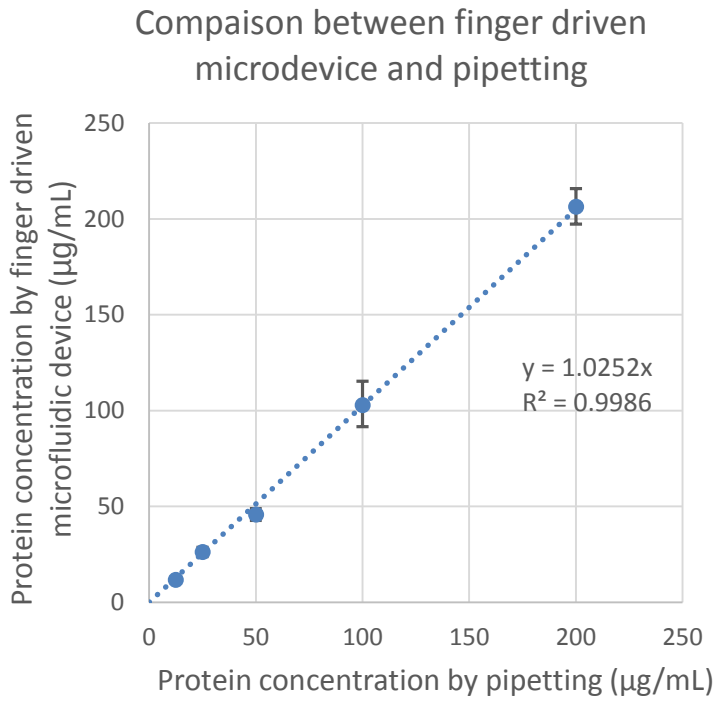


**Figure 8**. Colorimetric quantitation of protein using CBBG. The comparison between the protein concentrations determined by pipetting and finger-driven MaD(n=3).

### 3.7. Preparation of a PCR mixture on finger-driven microdevice

Having used dyes to demonstrate that metering of architecture-defined volumes was possible, we sought to demonstrate the accurate preparation of a biologically-relevant mixture. The polymerase chain reaction (PCR) is an enzyme-mediated amplification of select sequences of DNA in a reaction mixture that is cycled through temperatures that facilitate the denaturing of the template DNA (94 ℃), annealing of primers (~60 ℃) and

enzymatic synthesis of a copy of the sequence (72°C). The composition of this 'mixture' is important to the PCR chemistry and becomes increasingly so when multiple sequences are synthesized at the same time (multiplex PCR). The mixture that is thermally-cycled contains 4 different reagents - the polymerase (a), the DNA template (sample) (b), the PCR reaction mix (c) and DNase-free di$H_2O$ (d), with the a:b:c:d ratio varied slightly from PCR to PCR. Significant deviation from these relative ratios, especially on (c), can adversely affect the efficiency of the PCR because the PCR reaction mix contains dNTP and $Mg^{+2}$ and the efficiency of the PCR amplification is particularly sensitive to slight changes in $[Mg^{+2}]$.

The model PCR system we chose is a multiplex PCR that amplifies fragments short tandem repeat (STR) sequences) from 16 locations in the genome for use in human identification. With each location having the potential to present one or two different fragments, for any given sample, anywhere from 16 to 32 DNA sequences are amplified. The STR amplification is a finicky one, being very sensitive to the mass (pg) of DNA template (b) supplied, and the volume of PCR reaction mix (c) added. The a:b:c:d ratio required for the STR amplification is 1:11:21:22. As such, this represents a reasonable system to test the effectiveness of the finger-driven chip.

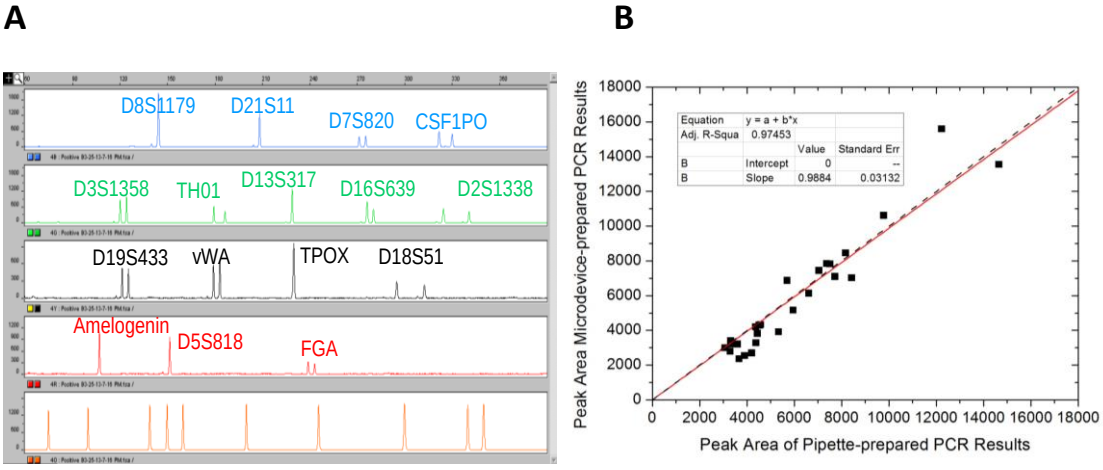


**Figure 9**. PCR results prepared by finger driven microdevice. (A) The electrophoretic STR profile of standard female DNA showing all 16 loci. The reaction mixture was prepared by the finger-driven microdevice; (B) Correlation of peak areas between pipetting-prepared STR-PCR (X-axis) and microdevice-prepared PCR (Y-axis)

Using the chip design and chamber combination that was described and validated in previous section 3.5, reagents were added to the chip and got MaD-ed, and the results are shown in Figure 9. Three consecutive cycles of MaD of combinations of reagents gave a total volume of 25 μL within 2 min. The correlation of peak area of all 16 loci between conventional pipetting-prepared PCR and chip MaD-prepared PCR is close to 1, proving that the biochemical reagents, especially those bio-macromolecules such as DNA and polymerase were not affected after processed by the microdevice and the PCR results are similar to those by the conventional method.

## CONCLUSIONS

We created a novel microdevice that can meter and deliver multiple solutions solely by finger pressing/releasing on only one finger pump, and the accuracy of the MaD on chip was proven to be comparable with pipetting. $CO_2$ laser ablation was used to achieve different thicknesses of the PDMS flap of the fluidic diode in the fast prototyping of the microdevice. The finger driven microdevice's utility was demonstrated through the preparation of a simple colorimetric test for protein quantitation, and a master PCR mix from four stock reagents. The single MaD fluidic circuit has been modularized to be structurally 'stackable', which enables easy multiplexing to achieve the MaD of arbitrary number of reagents without adding complexities in either fluidic structures or operations, which promotes the significance of our work not only in the growing of the concept of finger driven microdevices, but also in adding to the community of microfluidics a widely applicable designing reference for all the future single-pressure-source, passively-controlled muti-reagent handling microfluidic platforms.

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