



Correction: Virtual microwells for digital microfluidic reagent dispensing and cell culture

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Correction for ‘Virtual microwells for digital microfluidic reagent dispensing and cell culture’ by Irwin A. Eydelnant et al., *Lab Chip*, 2012, 12, 750–757, <https://doi.org/10.1039/C2LC21004E>.

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The authors regret that there was an error in Fig. 2C of the article. The units indicated for the virtual microwell number N_{vm} should be mm^{-1} rather than the m^{-1} that is indicated in Fig. 2C. A corrected version of Fig. 2C is given below. In addition, the threshold at which the dispensing behaviour changes is $N_{vm} = 20$, rather than the $N_{vm} = 2$ that is indicated in the text. To the authors’ knowledge, all of the raw data in the paper, as well as all other observations and interpretations in the paper, are correct.

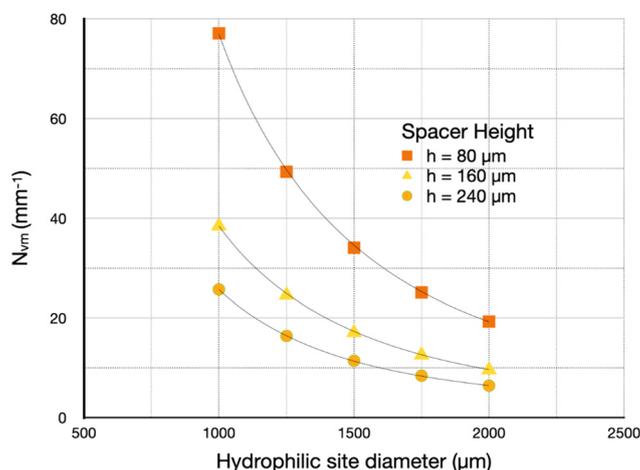


Fig. 2 (C) Plot of N_{vm} as a function of hydrophilic site diameter for “dry passive dispensing” experiments on digital microfluidic devices with inter-plate spacer heights of 80 μm (yellow circles), 160 μm (orange triangles), or 240 μm (red diamonds). In all cases tested, dry passive dispensing was successful for a single-unit source-droplet (*i.e.*, a droplet covering a single driving electrode) with $N_{vm} > 20$. For $N_{vm} < 20$, a double-unit source-droplet (covering two driving electrodes) was required for successful dry passive dispensing.

The authors thank James Wu from the Chinese University of Hong Kong for bringing this error to our attention.

The Royal Society of Chemistry apologises for these errors and any consequent inconvenience to authors and readers.

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Virtual microwells for digital microfluidic reagent dispensing and cell culture

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Digital microfluidic (DMF) liquid handling includes active (electrostatic) and passive (surface tension) mechanisms for reagent dispensing. Here we implement a simple and straightforward Teflon-AF lift-off protocol for patterning hydrophilic sites on a two-plate device for precise passive dispensing of reagents forming virtual microwells – an analogy to the wells found on a microtitre plate. We demonstrate here that devices formed using these methods are capable of reproducible dispensing of volumes ranging from ~80 to ~800 nL, with CVs of 0.7% to 13.8% CV. We demonstrate that passive dispensing is compatible with DMF operation in both air and oil, and provides for improved control of dispensed nano- and micro- litre volumes when compared to active electrostatic dispensing. Further, the technique is advantageous for cell culture and we report the first example of reagent dispensing on a single-plate DMF device. We anticipate this method will be useful for a wide range of applications – particularly those involving adherent cell culture and analysis.

Introduction

Miniaturization of laboratory procedures for lab-on-a-chip technologies requires on-device methods that are analogous to standard pipette-based reagent dispensing. This has been realized in microchannel-based systems through on/off device pumps,¹ in-line valves,² electrokinetic flow,¹ and capillary action,³ providing control of femto- to micro- litre volumes. In digital microfluidics (DMF), a technique in which droplets are manipulated across an array of insulated electrodes, active⁴ (electrostatic) and passive⁵ (surface tension) dispensing modes have been demonstrated. Here, we introduce an improvement to passive dispensing, with an emphasis on robustness and reproducibility, and applications in cell culture and analysis.

DMF devices are operated in either single or two-plate geometries. The single plate geometry typically consists of actuation electrodes with co-planar ground electrodes⁶ or a suspended grounded *catena*.⁷ In the two-plate format (Fig. 1A, 1B), a bottom plate is patterned with electrodes coated with a dielectric and hydrophobic material, and a top plate comprises a conductive layer coated with a hydrophobic material. A widely used function of two-plate digital microfluidics is active dispensing of droplets from reservoirs.⁴ As shown in Fig. 1C (panels i and ii), active dispensing is achieved by actuating a series of electrodes to stretch, neck, and pinch a droplet off

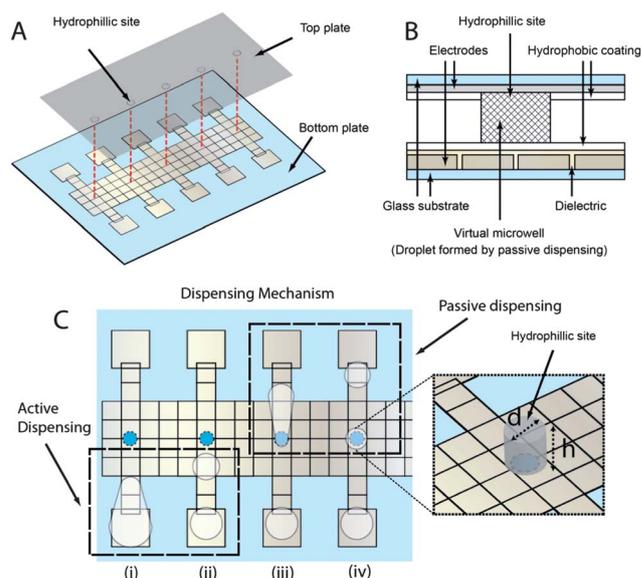


Fig. 1 Two-plate digital microfluidic (DMF) device design and assembly. (A) Exploded view of a device, comprising a bottom plate with patterned electrodes and a top plate bearing patterned hydrophilic sites. (B) Side-view, not to scale. (C) Schematic depicting two reagent-dispensing mechanisms on DMF. Active dispensing (i & ii) involves electrostatic stretching of a reagent from the reservoir followed by splitting. Passive dispensing (iii & iv) occurs spontaneously as a source droplet is translated across the hydrophilic site. The inset is a three-dimensional depiction of a virtual microwell, VM (*i.e.*, a droplet formed by passive dispensing). VM volume is dictated by the diameter of the hydrophilic site (d) and the distance between top and bottom plates (h).

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from a reservoir.⁴ Active dispensing highlights a particularly useful property of digital microfluidics: reagents and samples can be dispensed reliably and precisely on-demand. However, as demonstrated in this paper, inconsistencies arise when dispensing reagents with varying viscosities.

An alternative digital microfluidic function called passive dispensing was recently described by Barbulovic-Nad *et al.*,⁵ building on similar work by Chen *et al.*⁸ Passive dispensing is implemented using a DMF device surface that is primarily hydrophobic but patterned with hydrophilic regions. When a source droplet is translated across a hydrophilic site, surface tension effects result in spontaneous formation of a sub-droplet on the site (Fig. 1C, panels iii and iv). As described previously,^{5,9–12} passive dispensing is particularly useful for adherent mammalian cell culture, allowing for cell seeding onto dry hydrophilic sites, as well as for subsequent media and reagent exchange on droplet-bearing sites. We introduce here a new term for the cylinder-shaped droplet formed by passive dispensing: a virtual microwell (VM). The term VM is an analogy to the wells found on a microtitre plate. The “wells” described here are virtual as they are not confined on the sides like traditional wells, but are defined by the surface properties of the top and bottom plate. A similar strategy has been described previously¹³ for non-microfluidic applications, but this is the first time this concept is being applied within the context of DMF.

In initial work describing passive dispensing for cell culture,⁵ hydrophilic patches were formed by adsorbing extracellular matrix proteins onto Teflon-AF-coated DMF device bottom plate surfaces. Adaptation of this method for other applications realized several challenges, including: (1) inconsistent reagent dispensing both initially and during subsequent droplet passages, (2) protein dissolution and subsequent loss of hydrophilic pad integrity, and (3) difficulty functionalizing the electrode-bearing surface. Motivated by these challenges, we sought to develop a simple fabrication protocol for patterning hydrophilic sites directly on device surfaces.

Here we report a new method for forming hydrophilic patches relying on a Teflon lift-off procedure. The method is straightforward and fast, allowing for rapid generation of an array of individually addressable virtual microwells by passive dispensing. We demonstrate that this method can be used for two-plate DMF operation in air or oil to (1) reproducibly and precisely dispense reagents independent of viscosity based solely on device design parameters, (2) maintain constant droplet volume after subsequent reagent exchanges, and (3) improve cell seeding when compared with previously published methods. Further we show the first example of reagent dispensing on a single-plate device. We propose that these new methods will be useful for a wide range of applications – particularly those involving adherent cell culture and analysis.

Methods and materials

Reagents

Unless stated otherwise, general-use chemicals were from Sigma Aldrich (Oakville, ON, Canada) or Fisher Scientific Canada (Ottawa, ON, Canada), fluorescent dyes and cell media

components were from Invitrogen/Life Technologies (Burlington, ON, Canada), and photolithography reagents were from Rohm and Haas (Marlborough, MA). Deionized (DI) water had a resistivity of 18 M Ω ·cm at 25 °C.

Two-plate DMF bottom-plate fabrication

Digital microfluidic devices were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) cleanroom facility, using transparent photomasks printed at 20,000 DPI (Pacific Arts and Designs Inc., Markham, Ontario). Two-plate DMF device bottom-plates bearing patterned chromium electrodes were formed by photolithography and etching of commercially available chromium and positive photoresist coated glass slides (Telic, Valencia, CA). Briefly, substrates were exposed to UV through a mask (8 s, 29.8 mW cm⁻²) and then developed in MF-321 (~2 min). Chromium was etched in CR-4 (~5 min, OM Group, Cleveland, Ohio), and then substrates were washed with DI water, and dried under a stream of nitrogen. Substrates were then immersed in AZ 300T (3 min) to remove photoresist and then washed in DI water and dried under a stream of nitrogen. This was followed by cleaning in Piranha solution (10 s, 1 : 1 conc. sulfuric acid: 30% hydrogen peroxide). Substrates were rinsed in DI water, then dried under a stream of N₂, before dehydrating on a hot plate (165 °C, 10 min). As shown in Fig. 1A, the bottom-plate design featured an array of 116 actuation electrodes (2.2 × 2.2 mm each) connected to 10 reservoir electrodes (4 × 4 mm ea.), with inter-electrode gaps of 30–80 μ m. The actuation electrodes were roughly square with 140 μ m (peak to peak) sinusoidal interdigitations. In some experiments, the design also included an array of five 1 mm diameter optical windows (*i.e.*, circular regions free from chromium) with 9 mm between each window. Each window straddled the interface between two actuation electrodes. After patterning, the substrates were immersed for 30 min in silanization solution: 3-(Trimethoxysilyl)propyl methacrylate (Specialty Coating Systems, Indianapolis, IN), diluted to 1% (vol./vol) in 1 : 1 DI water : isopropanol. Substrates were air-dried for 30 min then washed with isopropanol (IPA) and dried under a stream of nitrogen. Substrates were then coated with 8 μ m of Parylene-C (Specialty Coating Systems) and 200 nm of Teflon-AF 1600 (DuPont, Wilmington, DE). Parylene-C was applied using a vapor deposition instrument (Specialty Coating Systems), and Teflon-AF was spin-coated (1% wt/wt in Fluorinert FC-40, 3000 rpm, 60 s) followed by post-baking on a hot-plate (165 °C, 10 min). Each driving electrode and reservoir was connected to a contact pad on the edge of the substrate. The polymer coatings were removed from contact pads by gentle scraping with a scalpel to facilitate electrical contact for droplet actuation.

For some experiments, hydrophilic sites were formed on ethanol sterilized DMF bottom plates as reported previously.⁵ Briefly, 2 μ L aliquots of fibronectin (33 μ g mL⁻¹ in DI water) were pipetted onto the Teflon-AF surface covering optical windows and were allowed to evaporate at room temperature for ~4 h. The adsorbed protein spots formed in this manner were roughly circular with ~1 mm diameter.

Two-plate DMF top-plate fabrication

Two-plate DMF device top-plates were formed from indium tin oxide (ITO) coated glass substrates (Delta Technologies Ltd, Stillwater, MN). For most experiments, ITO-glass substrates were coated with Teflon-AF and then were treated with a fluorocarbon lift-off procedure to form an array of hydrophilic spots (*i.e.*, circular regions of exposed ITO). Briefly, ITO-glass slides were immersed in RCA solution (6 : 1 : 1 DI water: 28% aqueous ammonium hydroxide: 30% hydrogen peroxide) for 15 min at 80 °C. After rinsing, drying, and dehydrating, substrates were spin-coated with Shipley S1811 photoresist (3000 RPM, 60 s) and then post-baked on a hot plate (2 min, 95 °C). The substrates were exposed (10 s, 29.8 mW cm⁻²) through a mask bearing an array of five 1.00-, 1.25-, 1.50-, 1.75-, or 2.00 mm diameter circular features (9 mm between each feature) and then developed in MF-321. After rinsing and drying, the substrates were flood exposed (10 s, 29.8 mW cm⁻²), and then spin-coated with Teflon-AF and post-baked using the same parameters used for bottom-plate substrates (*as above*). The substrates were then immersed in acetone with gentle agitation until the Teflon-AF over the patterned sites was lifted off (5–10 s). After rinsing and drying, the Teflon-AF was reflowed by baking on a hot plate at 165 °C, 210 °C, and 300 °C for 5 min at each temperature. For some experiments, unpatterned top-plates were formed without fluorocarbon lift-off, and were simply spin-coated with Teflon-AF using the same parameters used for bottom-plate substrates (*as above*).

Two-plate DMF device assembly and operation

Two-plate digital microfluidic devices were assembled with an ITO-glass top plate and a chromium-glass bottom plate as shown in Fig. 1B. The two plates were joined by stacking one, two, or three layers of double-sided tape (each layer ~80 μm), and were aligned such that the edge of the top plate was adjacent to the outer-edges of the reservoir electrodes on the bottom plate. In cases in which optical windows and top-plate hydrophilic sites were used, care was taken to align these features vertically (windows on the bottom plate and hydrophilic sites on the top plate). Driving potentials, ~300 V_{RMS} for operation in air or ~200 V_{RMS} for operation in oil, were generated by amplifying the sine wave output of a function generator (Agilent Technologies, Santa Clara, CA) operating at 18 kHz. Each reagent was loaded onto the device by pipetting an aliquot onto the bottom plate at the edge of the top plate, and simultaneously applying driving potential to the closest reservoir electrode (relative to the ITO electrode on the top plate) to draw the fluid into the reservoir. Thereafter, droplets were actively dispensed, moved, and merged by applying driving potentials to sequential actuation electrodes on the bottom plate relative to the ITO electrode on the top plate as described previously.⁴ In all DMF experiments, reagent solutions were supplemented with 0.02% Pluronic F68.¹⁴

Single-plate DMF device fabrication, assembly, and operation

The one-plate DMF device design consisted of twelve 3 × 3 mm square electrodes adjacent to a linear 1 mm wide ground electrode with 40 μm between each electrode. Devices were coated

with Parylene-C using the same method described for bottom plates of two-plate devices (*as above*). Devices were then coated with Teflon-AF bearing 1 to 2 mm diameter circular hydrophilic sites using a modified liftoff procedure. Briefly, 10 nm of chromium was deposited onto the Parylene by electron beam evaporation, which was then patterned into circular sites by photolithography and etching using parameters described for bottom plates of two-plate devices (*as above*). Devices were spin coated with Teflon-AF 1600 (1% wt/v in FC-40, 3000 RPM, 1 min), baked for 10 min at 165 °C then flood exposed (10 s, 29.8 mW cm⁻²). Devices were immersed in acetone with gentle agitation until Teflon-AF lifted off (~5–10 min) revealing a pattern of circular chromium features. Devices were rinsed with DI water, dried under a stream of nitrogen, then baked on a hot plate for 10 min at 165 °C.

Single-plate devices were loaded by pipetting 20 μL of reagent directly onto the outermost driving electrode. Sine wave driving potentials of ~600 V_{RMS} at 18 kHz were applied with the same amplified function generator described above. Droplets were made to translate across the device by potentiating sequential square electrodes relative to the linear electrode (held at ground) as described previously.⁶

DMF dispensing experiments

Devices bearing droplets were imaged with a CCD camera (Basler, Ahrensburg, Germany) mounted above the device. For two-plate devices, ImageJ software was used to estimate the apparent cross-sectional area of each droplet (typically circular but in some cases in the shape of an irregular polygon) and volume (knowing the intra-plate spacer thickness). For one-plate devices, each device was weighed on a microbalance before and after dispensing (after removal of the remainder of the source droplet with a tissue), allowing for estimation of dispensed volume on the basis of mass. The reagents evaluated included phosphate buffered saline (PBS) with 0.2% blue food dye, 0–65 wt% sucrose solutions prepared in DI water (with viscosities from literature values¹⁵), and Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS). At least three replicates were performed for all conditions.

In each experiment, an aliquot of the appropriate reagent was loaded into a device, and one of a number of conditions was evaluated. (1) *Active dispensing on a two-plate device*. A unit droplet (*i.e.*, a droplet covering one actuation electrode) was actively dispensed onto actuation electrodes and the volume was estimated. (2) *Passive dispensing onto top-plate hydrophilic sites in air on a two-plate device*. A unit droplet was actively dispensed as in (1) above and then translated over a hydrophilic site, and the volume of the passively dispensed droplet was estimated. In some instances, two unit droplets were actively dispensed and merged, then the combined droplet was translated over the hydrophilic site, and the volume of the passively dispensed droplet volume was estimated. For all spot sizes, dry dispensing (*i.e.*, passive dispensing onto sites not bearing a droplet), and wet dispensing (*i.e.*, passive dispensing onto sites bearing a droplet from a previous dispensing experiment) were evaluated. (3) *Passive dispensing onto top-plate hydrophilic sites in oil on a two-plate device*. Droplets were first passively dispensed in air (dry dispensing) as in (2), above. The entire device (*i.e.*, all of the space

between the top and bottom plates not occupied by a droplet) was then filled with light mineral oil. Wet dispensing was then evaluated as in (2), above. (4) *Passive dispensing onto bottom-plate hydrophilic sites in air on a two-plate device.* A unit droplet was actively dispensed as in (1) above, and then translated over the hydrophilic site (*i.e.*, a patch of adsorbed protein on the bottom plate), and the volume of the passively dispensed droplet volume was estimated. (5) *Passive dispensing on a one-plate device.* A 20 μL droplet was translated across a patterned hydrophilic site. A sub-droplet was generated by passive dispensing (after which, the volume was estimated), as the main droplet was actuated away.

Cell culture and experiments

Marbin Darby canine kidney (MDCK) epithelial cells were kindly provided by Dr N. Tufenkji (McGill University). MDCKs were cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin. Cells were incubated at 37 °C in a humidified incubator containing 5% CO_2 .

5 μL aliquots of cell suspensions (1×10^6 cells mL^{-1}) in media were pipetted onto reservoir electrodes of two-plate devices bearing optical windows on the bottom plate. Unit droplets were actively dispensed onto the electrode array, and translated across hydrophilic sites, (formed either by liftoff on the top-plate or fibronectin absorption on the bottom-plate), resulting in passive dispensing. Devices were then incubated at 37 °C in a humidified incubator containing 5% CO_2 for 18 h. Images of cells were acquired through the optical windows by light microscopy and the numbers of cells dispensed were enumerated using ImageJ.

Results and discussion

Lift-off patterning

DMF devices are typically coated with fluorocarbon (FC) polymers such as Teflon-AF (DuPont), CYTOP (Asahi), or Fluorad (3M). These materials have desirable properties including low surface energy, broad chemical resistance, thermal stability, and biocompatibility. Early DMF applications were implemented in devices bearing homogenous FC surfaces for applications including suspension cell culture,¹⁶ PCR,¹⁷ enzymatic assays,¹⁸ and DNA sequencing.¹⁹ More recently, DMF devices have been combined with heterogeneous surfaces (bearing different chemical functionalities) for more sophisticated applications. These methods can be sub-divided into those relying on modifications of the device surface itself⁵ or by incorporation of external materials with heterogeneous surface properties such as magnetic beads²⁰ or polymer plugs.²¹ In this paper, we focus on the former – heterogeneous patterned device surfaces. As described in the introduction (and depicted in Fig. 1C), DMF devices with surface modifications are particularly useful for a form of fluidic manipulation called passive dispensing.⁵

We report here a technique to form DMF devices that are globally coated with Teflon-AF, but periodically patterned with hydrophilic spots. In most of the work reported here, the hydrophilic spots were formed from exposed indium tin oxide (ITO) on the top plate of a DMF device as illustrated in Fig. 1B. Our new technique is similar to that described by Chen *et al.*⁸ and

Malic *et al.*²² for forming patterned Teflon-AF on ITO and gold surfaces, respectively. Significant trial and error was required to develop techniques that were reproducible with particularly important results being inclusion of an RCA cleaning step for improved adhesion of Teflon-AF to ITO and an extra UV exposure step to assist in photoresist removal. These measures and others (described in detail in the experimental section) form a robust and repeatable method that we have now used to pattern hundreds of substrates bearing circular structures with near-perfect pattern fidelity.

Passive dispensing and virtual microwells

The new methods for patterning surfaces described above were developed to facilitate robust formation of virtual microwells by passive dispensing.^{5,8} As shown in Fig. 2A, the simplest form of passive dispensing can be called “dry” passive dispensing, in which a VM is formed on an empty hydrophilic site. We evaluated the effects of varying gap spacing between the top and bottom plates and the diameter of the hydrophilic site on dry passive dispensing. As shown in Fig. 2B, by varying the hydrophilic site diameter from 1 mm to 2 mm and the inter-plate gap height from 80 μm to 240 μm , VMs with volumes ranging from ~ 80 nL to ~ 800 nL were formed. The precision of these volumes varied within the range of 0.7% to 13.8% for all conditions tested. The CVs increased with greater dispensed volume (either higher gap spacing or larger hydrophilic surface area).

In initial experiments, we observed that a single actively dispensed droplet (*i.e.*, Fig. 1C, frames i–ii) was not always sufficiently large to serve as the source droplet for dry passive dispensing. In such cases, the VM did not properly separate from the source droplet, or the remainder of the source droplet (after forming the VM) was too small to actuate away. Thus, for the conditions in Fig. 2B labeled with an asterisk (*), two droplets were actively dispensed and subsequently merged, and this combined volume served as the source droplet for passive dispensing. This observation led us to develop a quantitative criterion predictive of passive dispensing success, which we call the “virtual microwell number,” N_{vm} , which is defined in terms of the area of the square actuation electrodes (A_e), the area of the circular hydrophilic site (A_{hs}), and the distance between top and bottom plates (h):

$$N_{vm} = \frac{A_e}{A_{hs}h} \quad (1)$$

Fig. 2C summarizes N_{vm} across a range of device and feature parameters. We observe for all experiments where $N_{vm} > 2$, a single unit droplet actively dispensed from the reservoir was sufficient for successful generation of the VM.²³ For $N_{vm} < 2$, a single unit droplet was insufficient for successful passive dispensing. This is mostly consistent with the observations described by Chen *et al.*,⁸ with a discrepancy observed for cases when N_{vm} is close to 2. We propose that this discrepancy may be attributed to differences in device design and operation. Regardless, we anticipate that N_{vm} will be a useful heuristic in the design of VMs on DMF devices in the future.

For the majority of applications it is of interest to exchange reagents in VMs. We term this type of exchange “wet” passive dispensing, which is implemented when a source droplet is

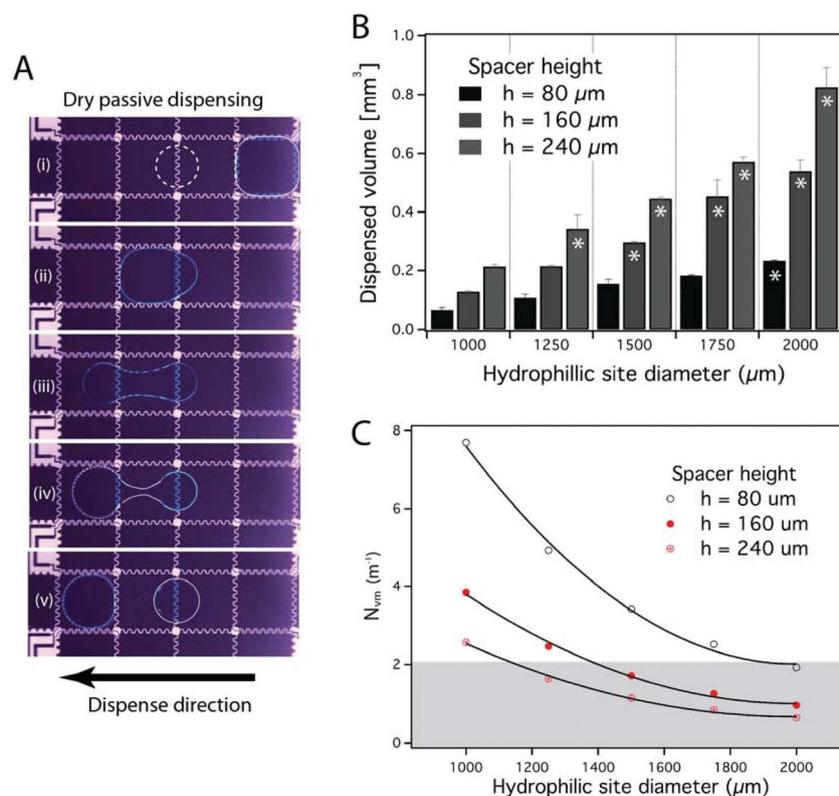


Fig. 2 Dry passive dispensing to form virtual microwells. (A) Video stills (top-to-bottom) depicting dry passive dispensing. The dashed circle in panel (i) indicates the position of the hydrophilic site. (B) Volumes of droplets dispensed in dry passive dispensing as a function of spacer height and hydrophilic site diameter ($n = 5$). Asterisks (*) indicate that source droplets were formed from two actively dispensed droplets. Error bars are 1 S.D. (C) Parameter N_{vm} calculated for each experimental condition in (B). The shaded region, $N_{vm} < 2$, indicates conditions in which two actively dispensed droplets were required to generate the source droplet for successful passive dispensing.

actively dispensed and then translated across a previously formed VM, displacing its original contents (Fig. 3A). Barbulovic-Nad *et al.*⁵ demonstrated that after three such exchanges, 100% of the content of the original VM is replaced. We evaluated VMs for hydrophilic sites with diameters ranging from 1000 to 2000 μm for the ability to repeatedly dispense volumes to sites bearing VMs. As shown in the gray bars in Fig. 3B, the precision in wet dispensing is very high for small sites, with CVs of 1.8%, 1.5%, and 0.7% for 1000 μm (126 nL), 1250 μm (196 nL), and 1500 μm (283 nL) diameter hydrophilic sites, respectively. Larger hydrophilic sites were associated with lower precision, with CVs of 12% and 7% for 1750 μm (385 nL) and 2000 μm (502 nL) diameter hydrophilic sites, respectively. Regardless, these data indicate that given volumes can be repeatedly dispensed to a given site multiple times with good (and for low volumes, excellent) precision.

The data above (and in most of the experiments described here) were generated using devices in which droplets were surrounded by a matrix of air. An alternative format is to fill devices such that droplets are surrounded by a matrix of oil, which has the benefit of lower voltage requirements, reduced surface fouling, and decreased droplet evaporation.²² We demonstrate here the implementation of passive dispensing in oil-filled DMF devices. Interestingly, we found dry passive dispensing in oil to be impossible. We speculate that this is because a thin film of oil film forms over the hydrophilic site and prevents hydrophilic

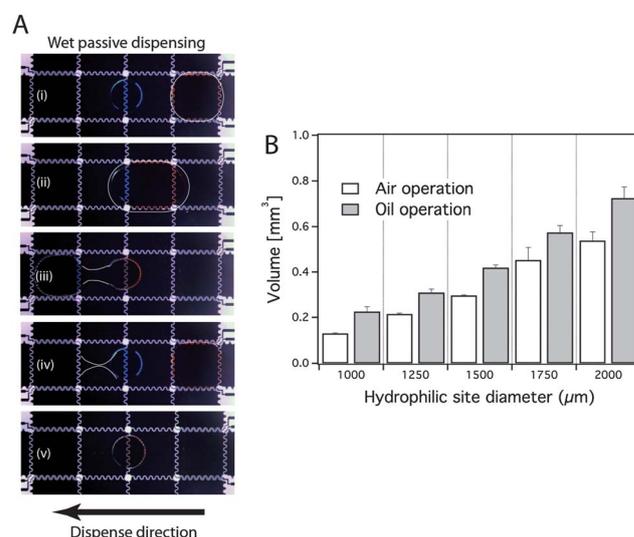


Fig. 3 Wet passive dispensing to exchange fluid in a virtual microwell. (A) Video stills (top-to-bottom) depicting wet passive dispensing in which the virtual microwell contained blue dye at the hydrophilic site (i) and a red dye source droplet is actuated across the virtual microwell displacing the blue droplet (ii–v). (B) Multiple passes of reagent across virtual microwells with varying diameters for 160 μm spacer height. The gray and white bars represent devices operated with a surrounding matrix of air and mineral oil, respectively. Error bars are 1 S.D.

interactions with aqueous droplets. In contrast, we found that wet passive dispensing in oil was straightforward when VMs were first loaded in air by dry passive dispensing and the devices were then filled with light mineral oil (which did not displace the aqueous droplets from the VMs) for wet dispensing, as shown in the white bars in Fig. 3B. The VMs formed in oil were in 1.1-fold to 1.25-fold larger the initial dry dispensed volume in air. We attribute this phenomenon to the increased viscous forces between the droplet and the filler medium.²⁴ With multiple passes, the oil-associated volume was maintained consistently, with CVs ranging from 2.8% to 9.4% for volumes of 240 nL to 810 nL. We propose that the capacity to combine passive dispensing with oil-filled devices will be useful for a range of different applications benefitting from the use of oil.

The data above (and in most of the experiments described here) were generated using two-plate DMF devices in which droplets are sandwiched between a top and bottom plate (Fig. 1). In the alternative single-plate device format, the larger droplet volume-to-electrode-area ratio results in lower actuation forces relative to two-plate DMF; thus as far as we are aware, there have been no reports of reagent dispensing (of any kind) on single-plate DMF devices. Here, we report the extension of the concept of passive dispensing on hydrophilic sites to single-plate DMF devices (Fig. 4). Applying fluorocarbon lift-off to single-plate DMF devices provides the ability to dispense reagents in this format. The hydrophilic sites in such systems were formed by evaporation of chromium (and subsequent patterning) on the top of the Teflon surface of a complete single-plate device. For hydrophilic sites with diameters of 1250 μm and 1500 μm , the droplets dispensed from 20 μL source droplets had volumes of 330 nL \pm 35 nL and 420 nL \pm 55 nL (CVs of 11 and 13%, respectively).

Active vs. passive dispensing

Active dispensing is regarded as the standard technique for reagent dispensing on DMF. In active dispensing, the liquid is stretched from a reservoir by electrostatic manipulation and then necked prior to splitting. Fouillet *et al.*²⁵ reported a CV of below 4% for active droplet dispensing of reagent into oil-filled DMF devices, similar to pipettes, where precision is reported at under 6% CV for 0.1 to 2.5 μL .²⁶ Actively dispensed volumes are limited by device geometry; different electrode sizes are required to achieve dispensing of different volumes.^{21,27} Further, when we examined the active dispensing of a range of sucrose solutions with varying viscosity from 0 cP to \sim 150 cP, we found poor repeatability, with errors of up to 30% and a 95% confidence interval across all viscosities of 0.2 mm^3 (red circles in Fig. 5). In

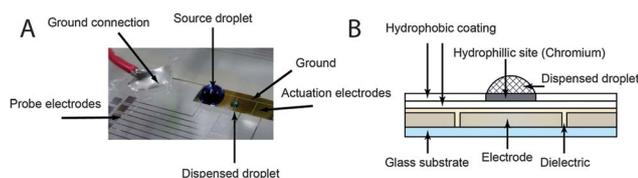


Fig. 4 Single-plate DMF passive dispensing. (A) Picture of a single-plate device depicting a source droplet and a passively dispensed droplet. (B) Schematic depicting the single-plate device geometry.

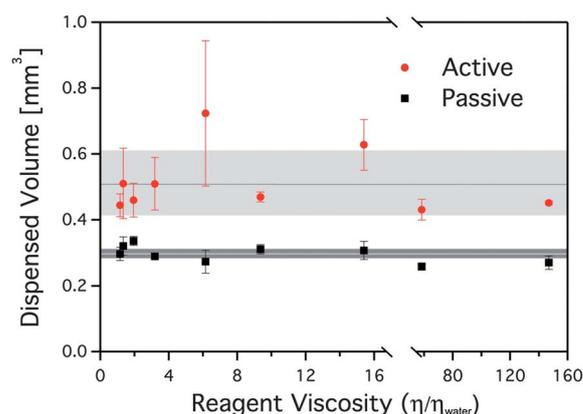


Fig. 5 Active and passive dispensing as a function of reagent viscosity. Sucrose solutions of varying viscosity were dispensed on DMF either by active or passive dispensing onto 1500 μm diameter hydrophilic sites ($n = 6$). Dispersed volumes are plotted as a function of solution viscosity. Error bars are ± 1 S.D., 95% confidence intervals are indicated by shaded regions, and the mean dispensed volume for each dispensing mechanism is indicated by a solid horizontal line.

comparison, VM volumes formed by passive dispensing were relatively independent of viscosity, with calculated 95% confidence intervals for dispensed volume of 0.02 mm^3 . In the future, we propose that the integration of different sizes of hydrophilic sites on device will make it straightforward to access a broad range of reagent volumes, and improve precision and accuracy of reagent dispensing independent of viscosity.

Lift-off vs. protein adsorption for passive dispensing

The liftoff-based techniques for forming hydrophilic patches on device top plates described here were developed as a result of our dissatisfaction with methods relying on hydrophilic patches formed from adsorbed proteins,⁵ for the reasons listed in the introduction. Here, we report a comparison of the two systems for the ability to passively dispense droplets containing suspended cells, and the ability of the cells to spread on the device surface. After performing independent trials of dispensing MDCK cells suspended in cell culture media, we found lower cell numbers and greater variability in the case of sites formed by protein spotting (cell number mean = 24 cells, cell number CV = 79%) as compared to those formed by fluorocarbon liftoff (cell number mean = 70 cells, cell number CV = 19%) (Fig. 6A). Furthermore, as shown in Fig. 6B, there were no significant differences in cell morphology for the two types of systems, which supports literature reports of ITO as being a suitable surface for cell culture.²⁸

In addition to improved reproducibility in dispensing, the new technique reported here has additional benefits for cell culture in DMF, including increased electrical isolation of cells from the actuation electrodes and compatibility with long-term culture. In DMF systems such as those reported here, the majority of the voltage drop occurs across the dielectric coating of the bottom-plate;¹⁶ however, localized charge densities at the device surface may vary, which might result in augmented transcriptional profiles within cultured cells. Further, localized heating in the dielectric layer might result in potentially deleterious cellular

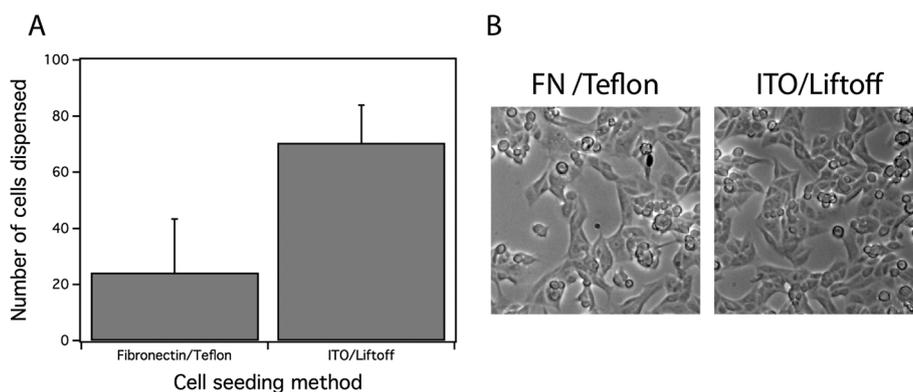


Fig. 6 Comparison of hydrophilic sites formed by adsorbed protein (on the bottom plate) vs. liftoff (on the top plate) for dispensing cells into virtual microwells. (A) Results of five trials seeding MDCK cells (5×10^5 cells mL⁻¹) by passive dispensing. (B) Bright-field images of MDCK cells seeded on fibronectin coated Teflon and indium tin oxide after 6 h. Scale bar = 50 μ m.

effects. Decoupling the cell culture site (by moving it to the top plate) from the electrode-bearing substrate (on the bottom plate) may dampen these effects. In the case of long-term culture, dielectric coatings are prone to failure due to accumulation of charge and moisture infiltration during incubation. Cell culture on the top-plate allows for the replacement of defective bottom-plates (with cells grown continuously on top-plates) without compromising the experiment being performed. We propose that this arrangement will be useful for the long-term culture of sensitive cell types, particularly stem and primary cells.

Conclusion

We present the utility of Teflon lift-off for improving digital microfluidic functionality. This precise method for patterning hydrophilic sites on hydrophobic DMF device surfaces resulted in multiple advances, including: (1) formation of virtual microwells for precise reagent dispensing, (2) passive dispensing in air and oil filled devices, (3) the first demonstration of passive dispensing on a single-plate device, and (4) improved surfaces for cell culture and other heterogeneous assays. We anticipate this new method will be useful for DMF-based techniques applied to a broad range of applications.

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