A chip-to-Chip nanoliter microfluidic dispenser

Jianbin Wang, ‡[a] Ying Zhou, ‡[a] Haiwei Qiu,[a] Huang Huang,[b] Changhong Sun,[b] Jianzhong Xi,*[b] and Yanyi Huang*[a]

[a] Department of Advanced Materials and Nanotechnology

College of Engineering, Peking University

Beijing 100871, China

E-mail: yanyi@pku.edu.cn

[b] Department of Biomedical Engineering

College of Engineering, Peking University

Beijing 100871, China

E-mail: jzxi@pku.edu.cn

‡ These authors contributed equally to the work.

Supporting Information

1. Microfluidic Dispenser Fabrication

We fabricated the microfluidic dispenser chips from poly(dimethylsiloxane) (PDMS) with multilayer soft lithorgraphy technology.

Single Layer Chip

First, we spin-coated a layer of SU-8 (Microchem, Newton, MA) 2050 photoresist (1700 rpm, 60 s, 100 μm) on a 3″ silicon wafer, to a thickness of approximately 100 μm, and baked it on a hotplate at 65ºC for 5 min and 95ºC for 8 min. The SU-8 was exposed to 300 mJ/cm^2 of collimated UV light (365 nm center wavelength) through a high-resolution transparency mask containing the design of the flow channels. The mask was printed at 20,000 dpi from an AutoCAD (AutoDesk, San Rafael, CA) design file. Then the SU-8 was baked at 65ºC for 3 min and 95ºC for

10 min, and then developed. The wafer was finally baked at 150ºC for 3 hours to fully crosslink the SU-8.

We treated mold with trimethylcholorosilane (Sinopharm, Beijing, China) vapor for 5 min at room temperature and poured on it uncured PDMS (RTV-615, GE Advanced Materials, Wilton, Connecticut, 5:1 ratio) to a thickness of 4 mm. After curing the PDMS by baking at 80ºC for 1 hour, the thick flow layer was peeled off the mold and punched for inlets on predefined locations, using a 20 gauge round hole cutter. These inlets were connected through Tygon tubing (0.020″ ID, 0.060″ OD, S-54-HL, Saint-Gobain Performance Plastics, Akron, OH, USA) with 23-gauge stainless steel tubes. Then the thick layer was bonded to a thin, cured, spin-coated layer of PDMS (20:1 ratio, 500 rpm 60s, ~200 μm, 80ºC for 1 hour) on a 3″ silicon wafer. We baked the device at 80ºC overnight and peeled the bonded PDMS layers off the flat silicon wafer. Before application, the chip was cut by a razor blade to form outlets. It is important to place the blanket layer face-up when cutting the chip to produce smooth edge.

Addressable Chip

The chip consists of two layers of PDMS, one with the channels where liquid flow occurs (flow layer), and the other with dead-ended channels that control the valves (control layer).

Both the molds for flow and control layer were made from AZ-P4620 positive photoresist (AZ Electronic Materials, Somerville, NJ, USA). We treated a 3″ silicon wafer with hexamethyldisilazane (Alfa Aesar, Ward Hill, MA, USA) vapor for 5 min at room temperature. Then we spin-coated the AZ photoresist onto the wafer (1000 rpm, 60 s) to a final thickness of 10 μm and baked at 65ºC for 3 min and 95ºC for 5 min. The photoresist was then exposed to 220 mJ/cm^2 UV light (365 nm) through a high-resolution transparency mask containing the design, and developed in AZ 400K developer (1:1 diluted by water). Finally, after soaked in water for 30 min to remove remnant developer, the photoresist was re-flowed and hardbaked on a hot plate ramped from 80ºC to 150ºC at 10ºC/hour, to obtain rounded patterns.

Before the PDMS chip fabrication, both molds were treated with trimethylcholorosilane vapor for 5 min at room temperature. We poured uncured 5:1 PDMS onto the control mold to a thickness of 4 mm to form the control layer. The flow layer was made by spin-coating uncured 20:1 PDMS onto the flow mold (2000 rpm, 60 s) . It then stayed horizontally for 20 min to form a uniform 35 to 40 μm thick layer. After curing the PDMS on the molds by baking at 80ºC for 1 hour for both layers, the thick control layer was peeled off its mold, punched for inlets and aligned over the fluid layer. Irreversible bonding of the two layers was achieved by baking them at 80ºC for 1 hour. After bonding, the layers were peeled off the flow mold, punched for fluid inlets and bonded to a thin, cured, spin-coated layer of PDMS (20:1 ratio, 500 rpm 60s, ~200 μm, 80ºC for 1 hour), by baking at 80ºC overnight. We finally peeled the device off the flat silicon wafer. Before application, the chip was cut by a razor blade to form outlets.

2. Glass Microwell Array Plate Fabrication

The microwell array was etched from a glass plate with a photo-lithographically patterned chrome (Cr) film. We found that the original positive photoresist (Shipley S1805) on the Cr film was fragile and may produce unwanted pinholes in the glass after wet etching. We removed the original photoresist with acetone and spin-coated SU-8 2010 (2000 rpm, 60 s, ~15 μm) onto the Cr film as a replacement and prebaked it on a hotplate at 65ºC for 3 min and 95ºC for 5 min. The SU-8 was exposed to 150 mJ/cm^2 365 nm UV, postbaked at 65ºC for 3 min and 95ºC for 5 min and then developed. The plate was finally baked at 150ºC for 3 hours to fully crosslink the SU-8.

The Cr film was patterned by a home made etchant (5 g $(NH_4)_2Ce(NO_3)_6$ and 4 ml concentrated nitric acid in 50 ml water). This layer of Cr was later used as etch-mask for glass (*Caution: the used etchant contains Cr^{6+} and is extremely toxic. It must be treated with reducer such as $FeSO_4$ before disposure*).

For protection, the backside of the glass plate was then coated with a layer of cured SU-8 before etching. Due to the isotropic etching process using a mixture of

hydrofluoric acid and hydrochloric acid ($HCl:HF:H_2O$ = 1:1:2), the diameters of the microwells in the glass plate increase as they go deeper. This effect had been taken into account in the mask design to compensate the diameter increase. After 2-hour etching, the microwells were in the shape of bowl with ~ 850 μm diameter and ~ 250 μm depth. The volume for each microwell is ~ 120 nl. The remaining SU-8 and Cr were removed by hot Piranha solution (a 3:1 mixture of concentrated H_2SO_4 and 30% H_2O_2 solution) and Cr etchant, respectively. (*Caution: Pirahna solution is very acidic and corrosive, and explosive when mixed with organic solvents. Mixing the solution is exothermic and it should not be stored in a closed container.*)

We used perfluorosilane (1H,1H,2H,2H-perfluorodecyltriethoxysilane, 97%, Alfa Aesar, Ward Hill, MA, USA) to modify the glass surface. 0.8 ml silane solution (5 μl silane in 2.5 ml toluene) was spin-coated (1000 rpm, 15 s) on a silicon wafer and then transferred onto a plat PDMS stamp for 5 min. We then stamped the PDMS onto the microwell plate and let it sit for 20 min for reaction. The contact angle of modified glass was 95°. This silane treatment is critical for a successful dispensation. After the contact transfer of silane, when we poured the aqueous solution onto the treated plate, it went straight into microwells. An untreated or uncompleted treated microwell plate would cause cross contamination between adjacent microwells.

3. Dispensation

We used a home-made CNC control box to drive a 3-axis moving stages, with a commercial control software (Mach3, Newfangled Solutions, Carmel, ME). We employed a digital I/O device (USB-6501, National Instrument, Austin, TX, USA) with a home-made driving circuit to control a solenoid valve (S15MM-30-12-3E, Pneumadyne, Plymouth, MN, USA) to switch compressed air on and off. A LabVIEW (National Instrument, Austin, TX, USA) program was also used to control the single channel addressable dispenser with integrated valves. The moving speed of the stages was 20 mm/s for X and Y axis and 10 mm/s for Z axis. Before the dispensation, we aligned the microwell glass chip and the PDMS microfluidic dispenser chip by adjusting the moving stages and two rotating stages. During the

dispensing process, we moved the dispenser chip directly above the wells and applied a pressure pulse (0.05 MPa, 18 ms) to drive the liquid through an 8-channel PDMS dispenser chip, and waited for 30 ms for the droplets to grow. Such parameter usually fills the microwells with about 115 nl liquid (variable with connection resistance and other conditions; calibration is needed if specific volume is desired). An even higher pressure (0.06-0.07 MPa) was applied to fill the microwells completely. For the single channel addressable dispenser, we applied a constant pressure in the fluid layer (0.06 MPa) and actuated the microvalves with a higher pressure (0.15 MPa). We controlled the addressable dispensing by releasing the pressure of the microvalves of certain channels through a LabVIEW program. Every single channel dispensation took 100 ms to complete the valve-switching and droplet generation. The slow-motion movies were taken with a high-speed CCD camera (Marlin F-033B, Allied Vision Technologies, Germany) at 200 frame/s and a digital camera (EX-F1, Casio, Japan) at 1200 frame/s and reedited to adjust the frame rate for viewing.

To investigate the dispensation range, we filled 128 microwells with both ferric thiocyanate ($Fe(SCN)_3$) and fluorescein using a 4-channel dispenser with different dispensation parameters. We measured the length of sample plug consumed in the Tygon tubing and calculated the volume assuming a uniform inner diameter. When we kept the pulse width constant and reduced the pressure to 0.01 MPa, we could filled the microwells with as little as 56 nl liquid. Even though our microfluidic dispenser could generate much smaller droplets when driven by shorter pulse, the microwells were too deep for the droplets to contact the hydrophilic inner surface and subsequently was trapped within the microwells. On the other hand, when we increased the pressure to 0.045 MPa, the dispensation could be as much as 170 nl., Our microfluidic dispenser could generate much larger droplets, but that would lead to overflow. Within the range between 56 – 170 nl using currently fabricated microwells, the best uniformity was achieved when the dispensation volume was ~ 115 nl with a coefficient of variation (CV) less than 6%. This condition was also used in the PCR experiments. The CV was higher when the dispensation volume reached the lower or higher limits of the microwell containers (CV ~ 14% for 56 nl and ~ 10%

for 80 nl and 170 nl) due to the physical shape of the wells. The dispensation volume beyond the current range (56 – 170 nl) could be achieved by changing the microwell chips with different well dimensions.

The dispensation variation was determined by filling the microwells with fluorescein, taking fluorescent microscopic images of each microwell with an Eclipse 80i microscope (Nikon, Japan) and integrating the fluorescence intensity through a home-written image process program. To prevent discrimination, we moved each microwell to the same location before taking a picture, so that the illumination intensity was equal. In the contamination examination, we rewrote the code of moving stage and the dispenser moved twice the pitches every time it filled the microwells (see the slow-motion movie #1). So they were alternatively filled with $FeCl_3$ and KSCN. Then we used the normal code to fill every microwell with water (see the slow-motion movie #2). No manual operation was needed during the 3-step reaction, ensuring the precision of the operation. We mixed $FeCl_3$ and KSCN solution in different ratio and observed the color as standard solution. We found that even 100 ppm of $FeCl_3$ in KSCN could produce the red product in the solution which was clearly observable, indicating that we were able to detect contamination above 100 ppm with this reaction system.

4. Polymerase Chain Reactions

We designed primers (71 bp amplicon) and a TaqMan probe (29 mer) for DsRed1 gene. All the oligonucleotides were synthesized by Invitrogen with sequences listed as following:

Forward primer	5’-GCAGCTGCCCGGCTACT-3’
Reverse primer	5’-CGATGGTGTAGTCCTCGTTGTG-3’
TaqMan probe	[FAM]-5’-CTACGTGGACTCCAAGCTGGACATCACCT-3’-[DAB]

pDsRed1 plasmids were amplified by DH5α cells and extracted using commercial kit (TIANprep Mini Plasmid Kit, Tiangen, Beijing, China). The 20 μl reaction system contained 100ng DNA, Taq PCR MasterMix (Tiangen, Beijing, China), 250 nM of each forward and reverse primer, 250 nM TaqMan probe, and

ddH_2O. Sample without template DNA was used as control 1, while sample without primers as control 2. In control 1 and control 2, the missing reagents were substituted by ddH_2O.

PCR experiments were carried out by a thermocycler (Dongsheng, Beijing, China) with a flat heating block. The thermal cycling protocol consisted of initial activation of the Taq polymerase (Tiangen, Beijing, China) at 95℃ for 5 min, followed by 35 cycles at 95 ℃ for 25 second and 60℃ for 75 second. The PCR results were examined by both 2% agarose gel electrophoresis and the fluorescence image analysis. The fluorescence micrographs were taken under a fluorescence microscope (Eclipse 80i, Nikon, Japan) by a CCD camera (DS-5Mv, Nikon, Japan). The intensity of a single well was extracted through image processing using Matlab (Mathworks, Natick, MA).

5. Slow-motion Movies

(1) Dispensing process of filling every alternative row of microwells. The movie was re-edited to play at 1/8 of the real speed. The movie was recorded with a Marlin F-033B CCD camera (Allied Vision Technologies, Germany).

(2) Dispensing process of filling every row of microwells. The movie was re-edited to play at 1/8 of the real speed. The movie was recorded with a Marlin F-033B CCD camera (Allied Vision Technologies, Germany).

(3) Dispensing process for parallel filling. The movie was re-edited to play at 1/10 of the real speed. The movie was recorded with an EX-F1 digital camera (Casio, Japan).