Supplementary Materials for

Turning On/Off Satellite Droplets Ejection for Sample Delivery on Digital Microfluidics

Haoran Li [1,2], Ren Shen [1,2], Cheng Dong [1], Tianlan Chen [1], Yanwei Jia [1,2,3,*], Pui-In Mak [1,2], Rui P. Martins [1,2,4]

Correspondence to: yanweijia@um.edu.mo

This PDF file includes:

Supplementary Text
Figs. S1 to S5
Tables S1
Legend for movies S1 to S4

Other Supplementary Materials for this manuscript include the following:

Movies S1 to S4
AutoCAD file: "Design of DMF chip"

Supplementary Note

DMF chip fabrication

Due to the unevenness of the thickness of the dielectric layer and the tilt of the chip platform, the drop may drift on the chip and move away from where it should be. In addition, the drop may move along the jetting bar that was charged for satellite droplets ejection, resulting in a decrease in the ejection amount. In our work, we fabricated square pillars of SU8, approximately 100 μm wide and 60 μm high, as fences on top of the 10 μm dielectric layer. Since the fences were located around the electrodes on the drop transportation path, the drop was prevented from drifting around and was restricted to the correct position (Fig. S2A).

The fabrication process is shown in Fig. S2B. DMF chips patterned with chromium (Cr) electrodes were firstly coated with 10 μm SU8-3010 as the dielectric layer by spin coating, followed by soft baking, UV exposure, post-exposure baking, development and hard backing. The electrode pad for connecting the DMF chip to the electronic control PCB was uncovered for electric conductivity. Then, another 60 μm thick layer of (SU8-3050) was coated on top of the dielectric layer followed by the same procedure described above to fabricate the patterned fences. Finally, the chip was coated with Teflon as a hydrophobic layer.

Calibration for the total volume determination of the ejected satellite droplets

Drops containing various concentrations of DNA fragments labelled with a fluorophore at the 3' end (Flr-only DNA) were loaded onto the DMF chip and mounted under a fluorescence microscope. The fluorescence images for each concentration were recorded, as shown in Fig. S4A. The fluorescence in each image was obtained by averaging the mean fluorescence in different regions in a drop and then averaged in replicated drops at various locations with the same sample concentration. The average fluorescence in dependence of the concentration was then plotted and fitted to a straight line (Fig. S4B) as the calibration line to determine the sample concentration from the fluorescence intensity.

For the calculation of the delivery volume of the on-chip pipette, a drop containing no fluorescent probe or a low concentration of the fluorescent probe picked up all the ejected tiny droplets and moved back and forth a couple of times to ensure homogeneous fluorescence distribution (Movie S4). Then, the average fluorescence was measured under the microscope and fitted to the calibration line to obtain the probe concentration in the drop. The probe in the delivered satellite droplets contributed the increase in the fluorescence of the picking drop. That gave:

$$V_j C_j = V_a C_a - V_i C_i \tag{1}$$

where V_j is the total volume of the ejected satellite droplets, C_j is the concentration of the ejected satellite droplets, V_i and V_a are the volume of the picking drop initially and after picking, respectively, and C_a and C_i are the concentration of the picking drop initially and after picking, respectively. Because the volume of the picking drop was about 0.5 μL, while the delivered volume was in the pL to nL range in our experiments, the volume change was negligible. V_i and V_a were the same as V_s, the picking drop volume. Therefore, the total volume of the ejected satellite droplets can be calculated as:

$$V_j = \frac{\Delta C \cdot V_s}{C_j} . \tag{2}$$

Jumping back of the satellite droplets to the dispensing drop

In a short duration ejection (0-3 secs), the volume of total satellite droplets increased linearly. However, the total volume of the satellite droplets began to change after continuous ejection for 3 sec, as shown in Fig. S5. We attributed this to the jump-back of some satellite droplets to the dispensing drop. Therefore, several ejections (each lasting 2 seconds) were performed in our experiments to minimize the effect of the jumping back phenomenon.

It should be noted that in order to ensure that the points on each data line were from the same ejection, the volume determination of Fig. S5 was different from the fluorescence method mentioned elsewhere in the work. Each ejection lasted for 10 seconds and the video was recorded, then the frame images of the same interval was processed to calculate the total volume of the satellite droplets using imageJ. The images of satellite droplets were taken by the camera mounted on the microscope. The processing of the images was illustrated in Fig. S6. The original image (Fig. S6a) was transformed from RGB color space to 8-bit grayscale for further processing (Fig. S6b). The grayscale image was then transformed to binary image (Fig. S6c). As shown in Fig. S6d, an edge detection method was applied to the binary image to find the outer contours of the droplets. The area of the droplets was determined by counting that geography pixel by pixel. False positives were filtered from the detected objects by their area and circularity. Fig. S6e illustrated the final detection overlapped with the original figure. The total jetted volume could be calculated through the following equation,

$$V = \Sigma_i \frac{4}{3}\pi\left(\sqrt{A_i/\pi}\right)^3$$

where A_i was the area of the i^{th} droplet and V was the total volume of the droplets. Here we regarded the shape of each satellite droplets as an approximate sphere. In fact, the droplet may not exist as a complete sphere on the plate. There may be some mist-sized droplets neglected in the image processing, and some droplets out of control of the image. However, for the same video, this off would exist in each image. So the calculated volume may not be the precise one as that obtained from the fluorescence method, but the trend of the total volume change in one ejection was meaningful, indicating the success of the delivery or the loss of some droplets.

Ejection volume calibration and calculation in DNA identification experiments

In the experiments for specific DNA identification, the delivery capability of the chip was pre-calibrated as described above using the Flr-only probe to minimize chip-to-chip variation. With a certain actuation signal (440 Vp, 800 Hz) and the same chip and same jetting bar width (100 μm), the delivered volume can be determined from the ejection time, as shown in Fig. S7A.

For the DNA identification of pathogens causing sepsis, the probe had a hairpin structure with a fluorophore and a quencher at the 5' and 3' ends. Therefore, no fluorescence emission was observed without a specific target. However, when the specific target was provided, the fluorescence linearly depended on the probe-target hybrid concentration. This allowed for another approach to double confirm the delivery volume by running similar fluorescence-concentration calibration, as shown in Fig. S7B. This result was regarded as the experimental measurement and compared with the expected volume calculated from the abovementioned calibration.

Robustness test of on-chip pico-dosing system.

In order to test the robustness of the "pico-dosing" chip, hundreds times of ejection at high voltage were run. In these experiments, satellite droplets were ejected by a fluorescent mother drop containing *Sulforhodamine B* (SRB) and picked up by a pick-up drop without initial fluorescence. The delivery volume was determined by the increase in fluorescence (same as mentioned above).

As can be seen in Fig. S8a, with our normal ejection conditions (800 Hz, 440 V, 2 seconds), the delivery volume was quite consistent till the fluorescence in the pick-up drop saturated after about 50 deliveries. Then, new mother droplet and pick-up droplet was replaced, and ran 5 rounds (158 deliveries in total) tests on the same chip and same electrode. Fig. S8b shows that the linearity remains in the following a couple of rounds of tests. That indicated that after around 150 deliveries, the system was still good at consistent delivery. In order to reflect the continuous performance, we reduced the voltage (325V) and mother droplet concentration (50 μM) to avoid the pick-up droplet's fluorescence intensity from reaching saturation prematurely, so that more deliveries can be performed. Fig.S8c shows that in 120 deliveries, the linearity is still very good. And considering that 150 ejections had been carried out before this round of test, the chip has tolerated a total of around 300 deliveries with consistent performance. These experiments prove that the chip is robust enough for long-term use.

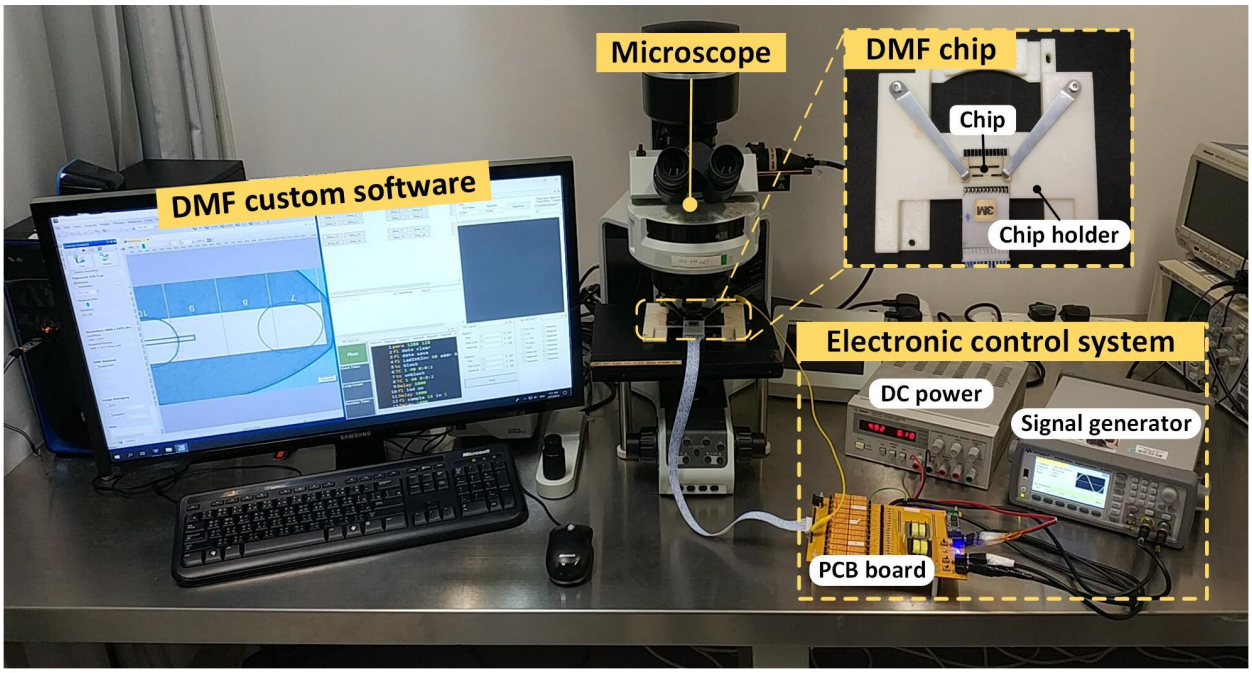

Fig. S1. DMF system setup. The DMF system consisted of four parts: the DMF chip on the chip holder, the electronic control system including the DC power supply, signal source and PCB board, the fluorescence microscope with control software, and custom software for DMF manipulation.

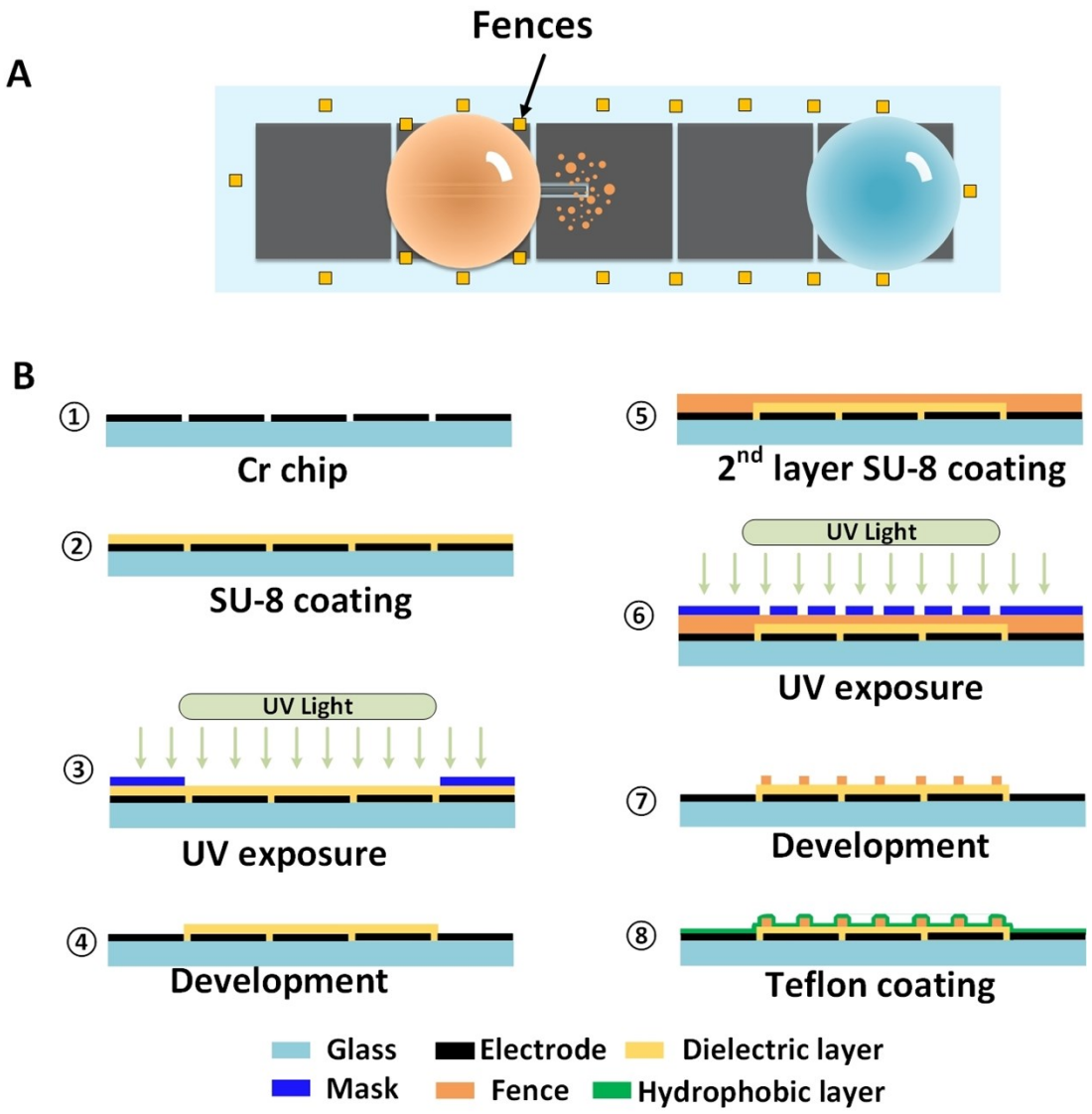

Fig. S2. SU8 fences on a DMF chip and chip fabrication procedures. (A) The position of fences on the chip. (B) The fabrication process of the DMF chip.

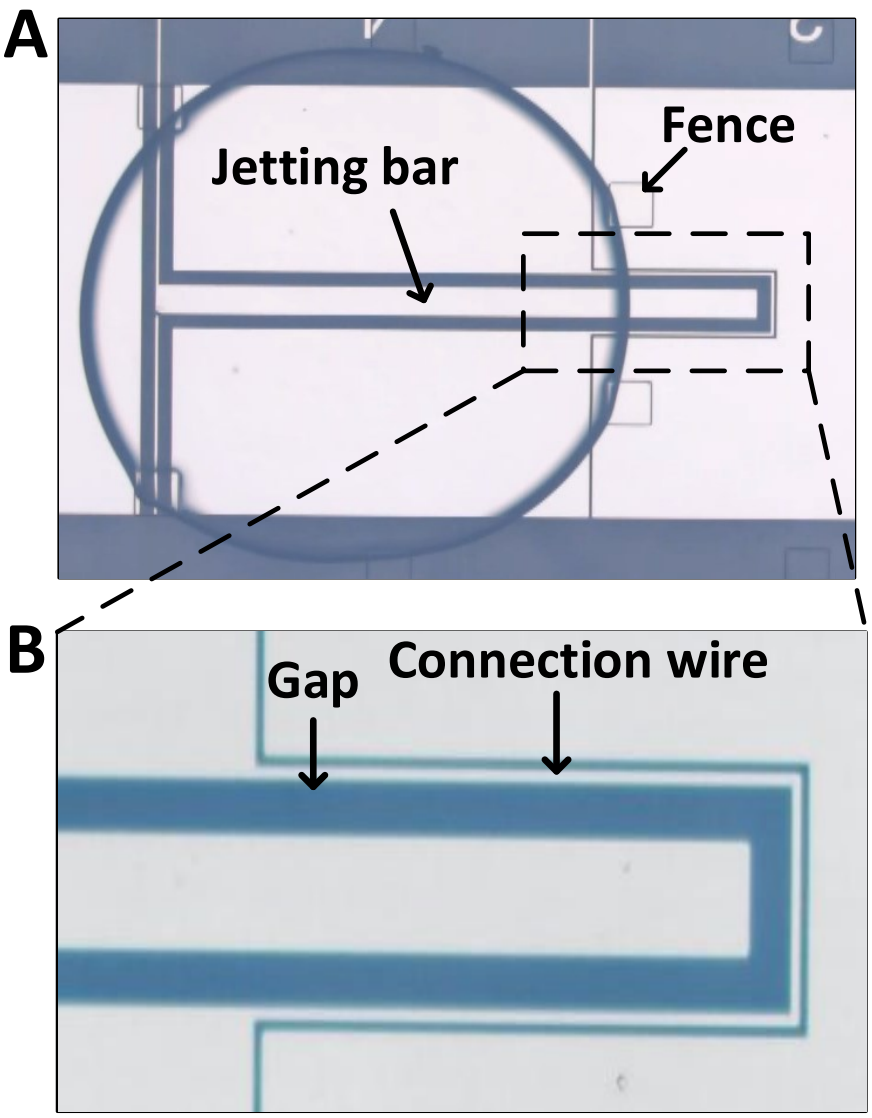

Fig. S3. The structure of "pico-dosing". A) Electrodes layout. B) The structure of jetting position

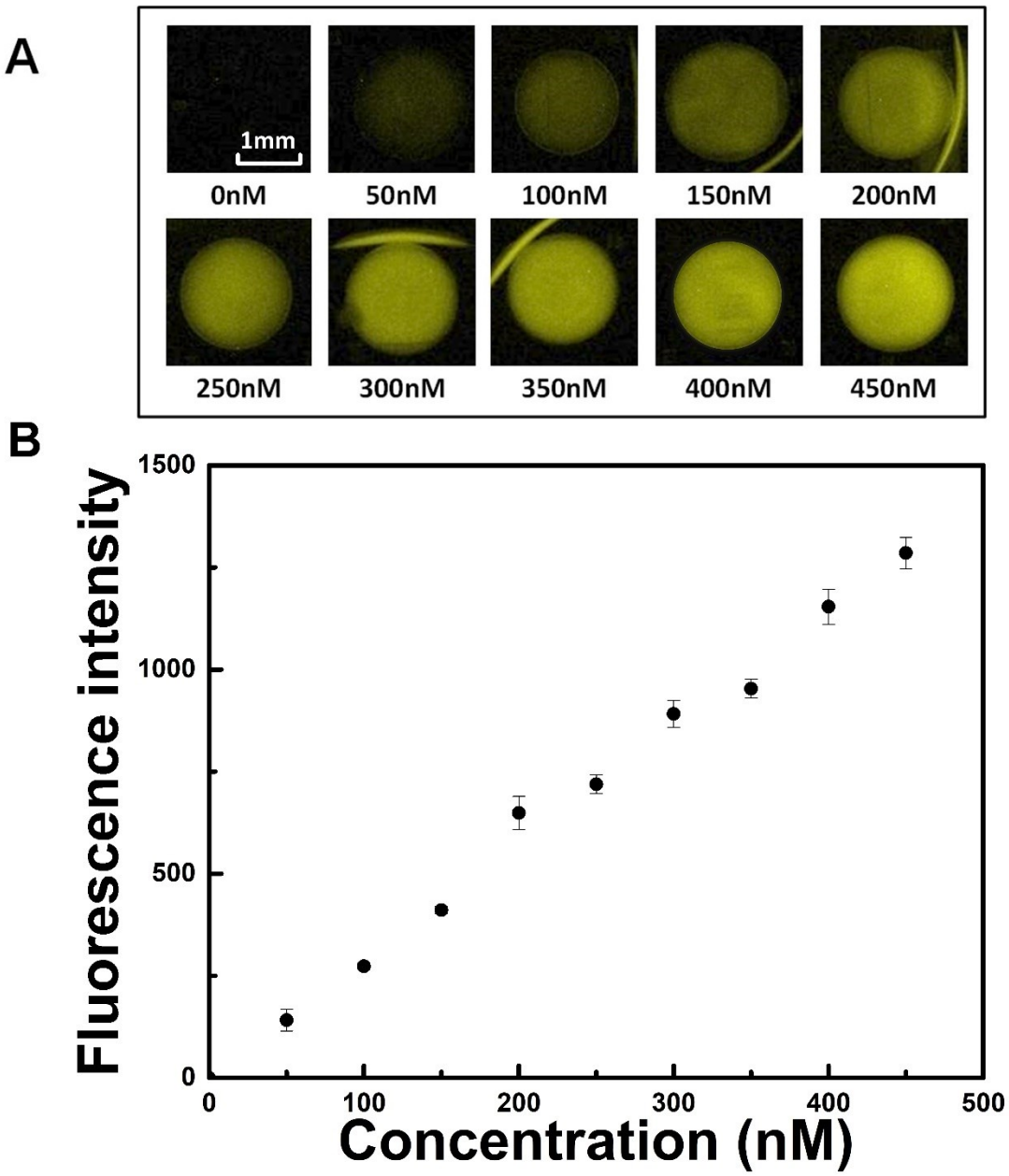

Fig. S4. Calibration of the fluorescence for delivered volume determination. (A) Fluorescent images of drops with various fluorescent DNA probe concentrations. (B) The calibration line of the fluorescence intensity in dependence of the probe concentration.

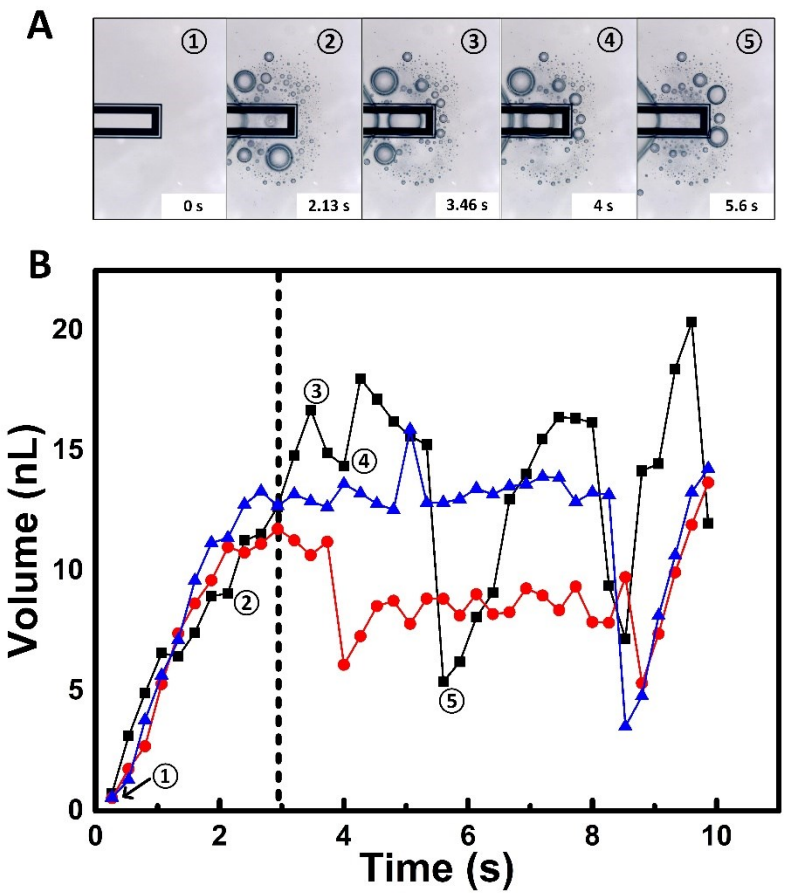

Fig. S5. The "jump-back" during injection. A) A series of images of the process of jetting and the "jump-back" phenomena. B) Effect of the actuation time on the delivered volume.

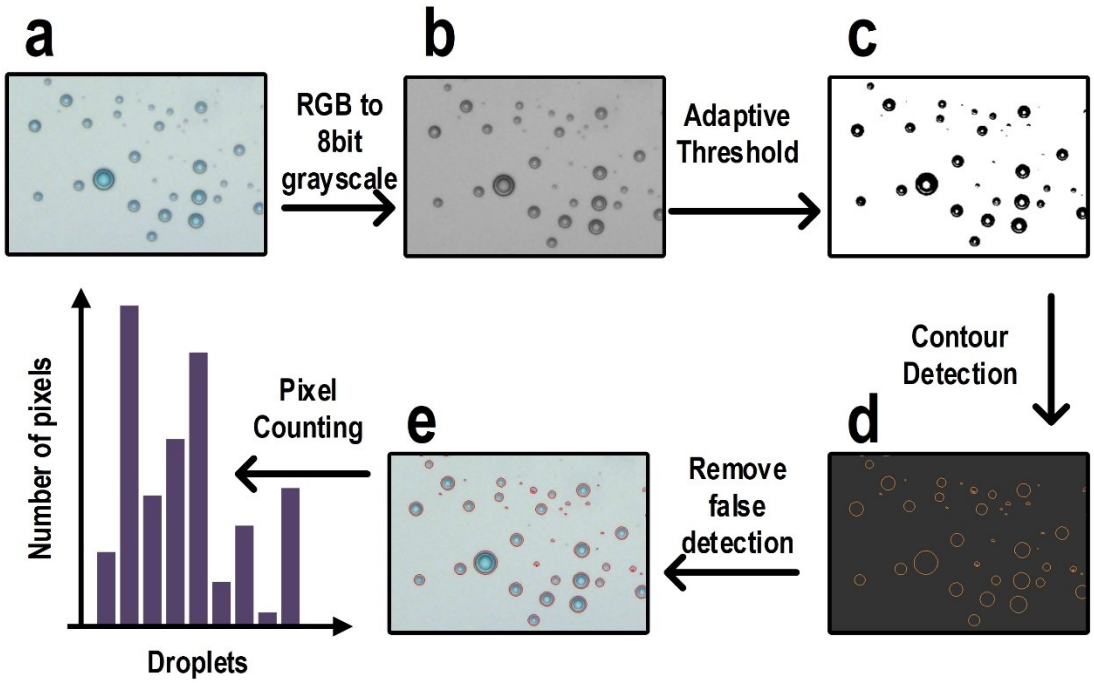

Fig. S6. Image processing of volume determination of satellite droplets.

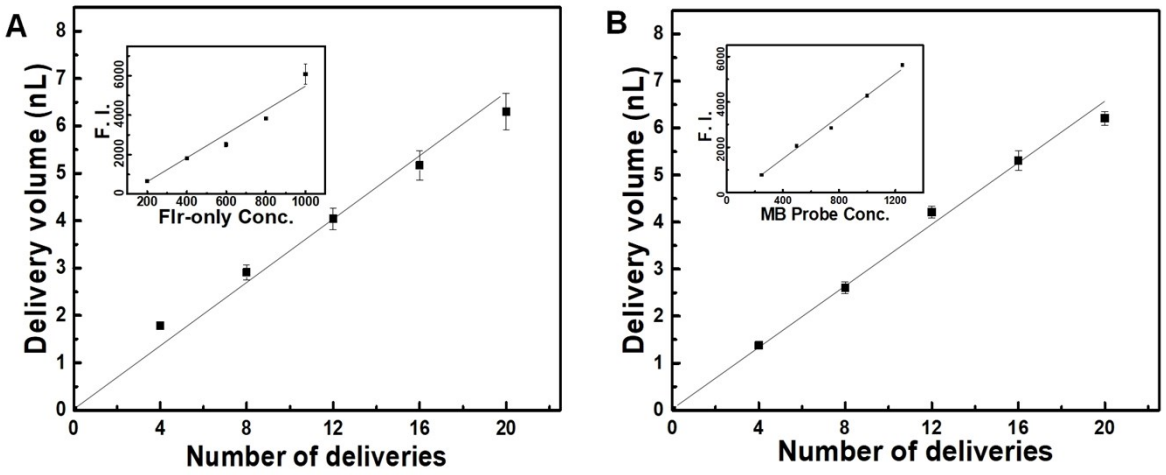

Fig. S7. Delivery volume in specific DNA identification. (A) Pre-calibration of the chip and actuation system setup to obtain the expected delivery volume from control parameters. (B) Calibration of the chip in a probe-target hybridization experiment to obtain the experimental measured volume.

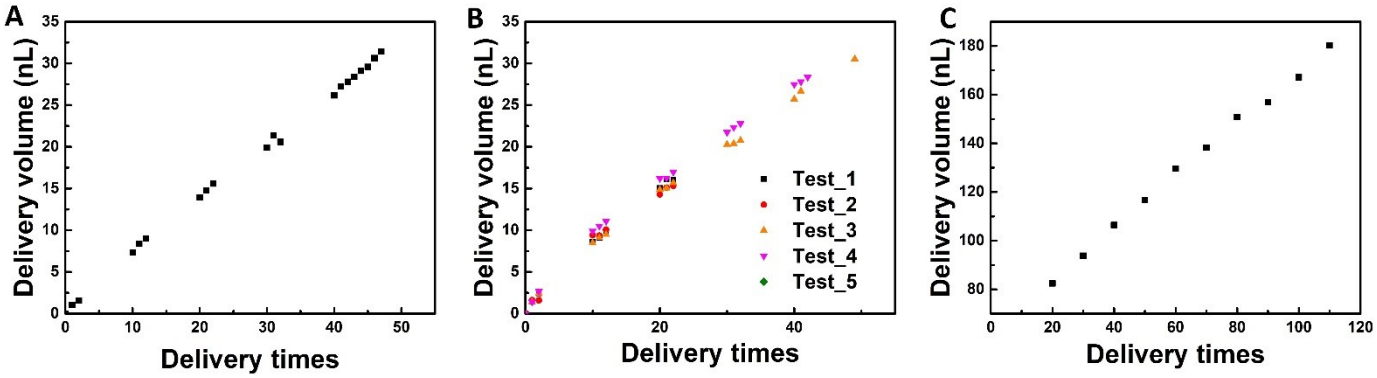

Fig. S8 Robustness test of the pico-dosing chip. (A) A round of 47 deliveries test. (Vp = 440V, frequency = 800Hz) (B) 5 rounds (158 times in total) of delivery tests. (Vp = 440V, frequency = 800Hz) (C) A round of 110 deliveries test at 325V (Vpp) and 800Hz driving voltage.

Table S1. DNA identification experimental design

	Dispensing drop	Picking drop
Pre-test	100 μM flr-only DNA probe	10 mM Tris-HCl
Formal test	100 μM MB probe (S. Aureus)	10 μM *S. aureus* target
		10 μm *K. pneumoniae* target

Movie S1. Drop transportation on DMF chip.

Movie S2. Satellite droplets ejection under high voltage alternating electric field.

Movie S3. On-chip sample delivery demonstrated by a pH indicator.

Movie S4. Ejection and delivery of sample containing fluorescent probe.