Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Centrifugal Micro-Channel Array Droplet Generation for Highly Parallel Digital PCR

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1. Centrifugation process

The process of centrifugation includes three stages in terms of rotating speed: speeding-up, steady spinning, and braking. As is demonstrated in **Figure 3**, the size of droplet closely linked to the rotating speed. Thus in order for a uniform and monodisperse micro-emulsion, one should avoid droplet generation in speeding-up and braking stages so that most of droplets are generated under steady rotating speed. Extending set centrifuge duration is a feasible method for droplet generation such that the aqueous liquid would be exhausted before entering the braking stage (**Figure S1**). The accelerating stage is nonetheless inevitable. To yield emulsion droplets with better monodispersity we investigated the number of the microchannels in a single MiCA plate. By reducing the number of the channels, the overall flow rate is reduced so that the proportion of the fluid flowing during accelerating stage decreases. When the set acceleration is above 10,000 g, our centrifuge machine only takes 10 seconds to reach full speed, leaving more than ~98% sample becoming droplet during steady spinning stage.

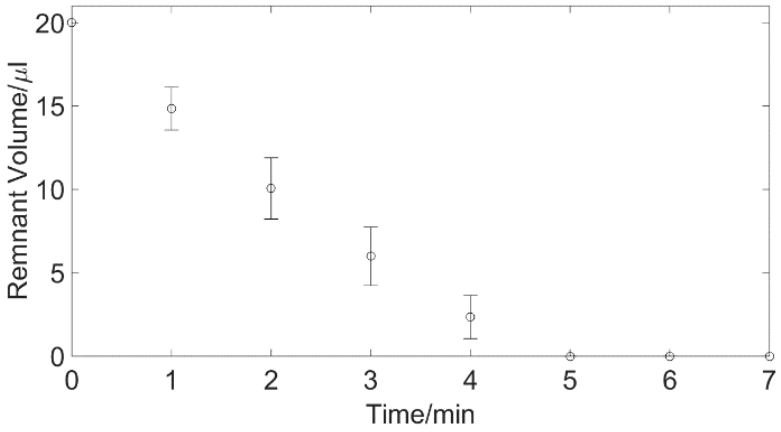

Figure S1. Remnant volume of 20 μl sample, spun at 130,000 $m \cdot s^{-2}$ for different time.

2. MiCA plate surface treatment

The surface hydrophobicity treatment of the glass slices included acid washing with Piranha solution (*CAUTION: Piranha solution is extremely energetic and may result in explosion or skin burns if not handled with extreme caution!*) oxygen plasma activation, perfluoroalkyl silane vapor deposition and isopropyl ethanol washing. We submerged the MiCA plates in Piranha solution prepared beforehand and subjected them to ultrasonication for 10 min. Having been washed with DI water and dried under 70 ℃ for 20 min, the MiCA slices were put into an oxygen plasma cleaner (Chengdu Mingheng PDC-MG) for surface activation (5.5 min, 890 V, 220 mA, 7.0 Pa). Then we transferred the activated glass slices into a vacuum desiccator, which contains an open vial with 200 μl 1H,1H,2H,2H-perfluorooctyltrichlorosilane (PFOTCS) (Sigma). We later vacuumed the desiccator to less than 0.1 psi for 50 min. Subsequently, we heated the MiCA on a hotplate for 5 min at 120 ℃. We finally washed the MiCA plates with isopropyl ethanol (MOS grade) and then again with DI water, dried them in ambient environment.

3. Digital PCR assays

The oligonucleotide sequences are listed in **Table S1**. The target DNA was first chemically synthesized and confirmed by Sanger sequencing and then PCR amplified. The prfA sequence was first PCR amplified with forward and reverse amplification primers (Q5® High-Fidelity 2X Master Mix, 95℃ 2 min activation, 32 cycles of 94℃ denaturing 15 seconds, and 60℃ annealing and extension 30 seconds) and then purified with agarose-gel electrophoresis. Recovered DNA concentration was roughly determined by Qubit and later serially diluted while the final concentration was validated by Bio-Rad QX200 dPCR platform.

Table S1. Oligo DNA sequences

Oligo-DNAs	Sequence
prfA gene 280-bp fragment (template)	CCGCAAATAGAGCCAAGCTTCCCGTTAATCGAAAAATCATTAAATT TAGCTAGACTGTATGAAACTTGTTTTTGTAGGGTTTGGAAAACATA GAAAAAGTGCGTAAGATTCTTGCTCAGTAGTTCTTTTAGTTCGTTT ATTTTGATAACGTATGCGGTAGCCTGTTCGCTAATGACTTCTAAAT TATAATAGCCAACCGATGTTTCTGTATCAATAAAGCCAGACATTAT AACGAAAGCACCTTTGTAGTATTGTAAATTCATGATGGTCCCGTTC TCAC
Forward primer for template production	5’-CCGCAAATAGAGCCAAGCTT-3’
Reverse primer for template production	5’-GTGAGAACGGGACCATCATG-3’
Forward primer for TaqMan assay	5’-GCCTGTTCGCTAATGACTTCTAAAT-3’
Reverse primer for TaqMan assay	5’-GTGCTTTCGTTATAATGTCTGGCTTT-3’
TaqMan probe	FAM-5’-TAATAGCCAACCGATGTTT-3’-MGB

Table S2 lists the recipe of dPCR premix. The mixture was then loaded into MiCA tubes, and spun under 130,000 $m \cdot s^{-2}$ for 7 minutes leading to the liquid samples becoming 52.5 μm droplets with no dead volume. The emulsion droplets then went through two-step PCR thermocycling: 25 °C 10 min for surfactant encapsulation, 95 °C 2 min for enzyme activation, 40 cycles ramping (15 s at 94 °C and 30 s at 60 ° C) for amplification.

Table S2. Recipe of 2X premix for MiCA dPCR.

For 50 μl premix	Vol./ μl	Stock Conc.	Final Conc.
Polymerase Buffer †	10	10X	2X
$MgCl_2$ †	10	50 mM	10 mM

dNTP	4	10mM each	0.8 mM each
Forward Primer	5	20 μM	2 μM
Reverse Primer	5	20 μM	2 μM
TaqMan Probe	5	6 μM	600 nM
Platinum™ Taq Polymerase	1	10 U/μl	0.2 U/Rxn
Nuclease free water	10	--	--

† Both are from Platinum™ Taq polymerase kit

4. Digital counting of droplets

We modified the flow-chamber of a flow cytometer (BD FACS Jazz) to detect the droplets, both the fluorescent (Taqman probe positive) and non-fluorescent (Taqman probe negative) ones. Emulsion droplets were pumped into an inlet of a T-junction along with diluting oil into the other inlet (**Figure S2**). The diluted emulsion then flowed through a borosilicate glass micropipette with a tapering section in middle. A 488 nm laser was used for excitation. The fluorescence signals are collected by a PMT which voltage are measured by a high-speed DAQ card (National Instruments myDAQ).

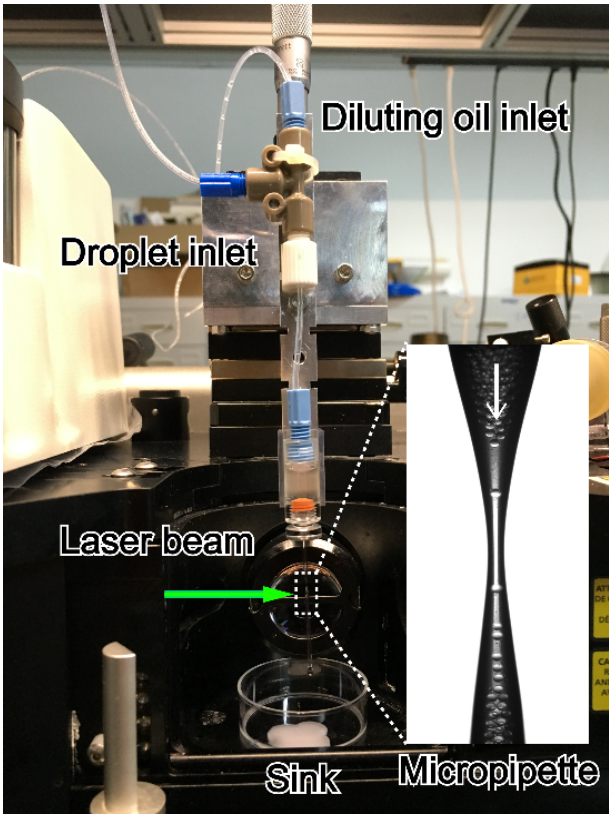

Figure S2. Modified flow chamber for droplet counting using a flow cytometer.

Taper fabrication: The borosilicate micropipettes (Sutter B100-30-7.5HP) were pulled using a capillary puller (Sutter P-1000). After 11 cycles of heat-pulling (Heat 500℃, Pull 2 times, Velocity 2, Time 5 s, Pre 500℃, Ramp 500℃), a tapered neck was formed with internal diameter ~100 μm.

5. Signal Analyze

We use MATLAB to analyze the signal captured by the DAQ card. Our program finds the pulses of the fluorescence signal. Each pulse indicates one droplet flowing through the detecting area, whose height is taken as the fluorescence intensity of the droplet. The distribution of fluorescence intensity of all the droplets in one experiment is shown in the Figure S3. As there were very few signals that fell between the two main peaks of the histogram, a threshold was set to 2 V to assign the 'positive' and the 'negative'.

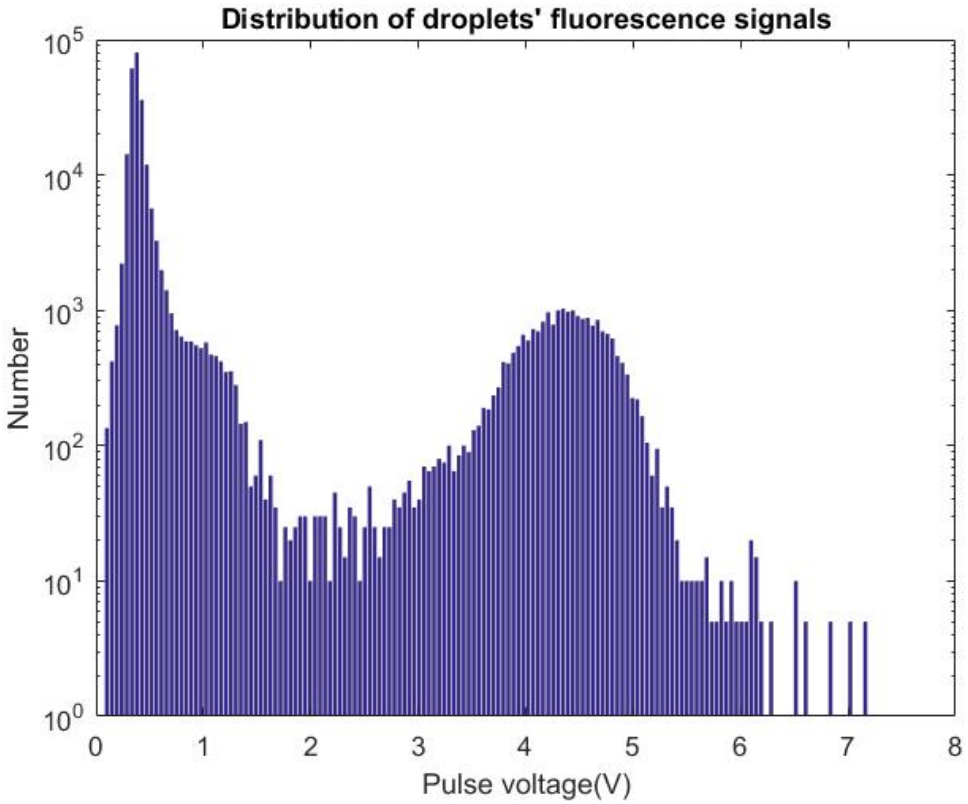

Figure S3. Pulse voltage distribution of dPCR droplets in a typical sample.

6. MiCA dPCR data analysis

Aqueous sample of volume *V* is divided into *N* partitions, average volume of each partition $\bar{v}$, and *m* copies of DNA template are dispensed in it. After Poisson process, *n* partitions/droplets signal positive. Define

$$p \stackrel{\text{def}}{=} \frac{n}{N} \qquad \text{Eq S1}$$

$$\lambda \stackrel{\text{def}}{=} \frac{m}{N} \qquad \text{Eq S2}$$

The purpose is to determine λ thus to get template concentration

$$C \overset{\text{def}}{=} \frac{m}{V} = \frac{\lambda N}{V} \quad \text{Eq S3}$$

Of a given droplet, the probability of having at least one template is

$$\rho = 1 - (1 - \frac{1}{N})^m \quad \text{Eq S4}$$

And when *N* is great enough,

$$\rho \approx 1 - e^{-\frac{m}{N}} = 1 - e^{-\lambda} \quad \text{Eq S5}$$

Thus for *N* droplet, the positive number *n* follows binomial distribution $n \sim \mathrm{B}(\rho, N)$, whose expectance and variance are

$$\mathrm{E}(n) = N(1 - e^{-\lambda}) \quad \text{Eq S6}$$

$$\mathrm{D}(n) = Ne^{-\lambda}(1 - e^{-\lambda}) \quad \text{Eq S7}$$

And for $p = \frac{n}{N}$,

$$\mathrm{E}(p) = 1 - e^{-\lambda} \quad \text{Eq S8}$$

$$\mathrm{D}(p) = e^{-\lambda}(1 - e^{-\lambda})/N \quad \text{Eq S9}$$

Therefore

$$\lambda = -\ln\left[1 - \mathrm{E}(p)\right] \quad \text{Eq S10}$$

We use p as an unbiased estimate for $\mathrm{E}(p)$, therefore it can be estimated that

$$\hat{\lambda} = -\ln\left(1 - p\right) \quad \text{Eq S11}$$

In the practice of MiCA dPCR, we examine *N*' droplets out of population *N*, and n' among *N*' droplets are found positive. The effective droplet rate *κ* can be expressed as follows.

$$\kappa = \frac{N\prime}{N} \quad \text{Eq S12}$$

Since in our experiment, effective droplet rate *κ* is above 80%, it is reasonable to assume

$$\frac{n}{N} = \frac{n\prime}{N\prime} \quad \text{Eq S13}$$

thus

$$\hat{\lambda} = -\ln\left(1 - \frac{n\prime}{N\prime}\right) \quad \text{Eq S14}$$

In the practice of dPCR, *N* is also an estimate. To estimate *N*, one should either count all the droplet to have the exact value of *N*, or assume all the droplets are perfectly monodispersing, thus

$$N = \frac{V}{\bar{v}} \quad \text{Eq S15}$$

$$\hat{C} = -\ln\left(1 - \frac{n\prime}{N\prime}\right)\frac{1}{\bar{v}} \quad \text{Eq S16}$$

Where

$$\bar{v} = \frac{4\pi r^3}{3} \quad \text{Eq S17}$$