Supporting Information for

A Large-scale Pico-Droplet Array for Viable Bacteria Digital Counting and Dynamic Tracking based on Thermosetting Oil

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Supplemental method section S1:

Stochastic confinement of single bacteria in pico-droplets

In the process of sample partition, the bacterial cells are randomly distributed to pico-droplets, in which the cell number is fitting Poisson distribution. The probability of cell number in a pico-droplet can be calculated according to the Poisson distribution formula:

$$P(k,\lambda) = (\lambda^k \cdot e^{-\lambda})/k! \quad (1)$$

Where k is the number of bacterial cells in pico-droplets (0, 1, 2, 3…) and λ is the average number of bacterial cells per pico-droplet, P means the probability that there are k cells in pico-droplet. When the bacterial sample is dispersed by the pico-droplets, there is at least one cell in the pico-droplet, thus the Poisson distribution formula can be expressed as:

$$\mathrm{P(k>0)} = 1 - \mathrm{P(k=0)} = 1 - e^{-\lambda} \quad (2)$$

The chance of having cells in pico-droplets also equals the proportion of positive pico-droplets, then

$$P(k>0) = 1 - e^{-\lambda} = positive/total \quad (3)$$

positive represents the count of positive pico-droplets and *total* is the number of pico-droplets on chip.

Besides,

$$\lambda = \mathrm{C} \cdot \mathrm{V}$$

C represents the concentration of bacterial cells and V is the volume of per pico-droplet.

The formula (3) can be rewritten as:

$$1 - e^{-\mathrm{C}\cdot\mathrm{V}} = positive/total \quad (4)$$

Thus

$$C = -\ln(1 - positive/total)/\mathrm{V} \quad (5)$$

Because of random and independent distribution of bacterial cells, the number of positive droplets is not same as that of cells. The effect of Poisson distribution is converting the positive droplets number into the exact number of bacterial cells in each sample condition. Therefore, the accurate concentration of bacterial sample can be calculated.

Supplemental Tables:
Table S1

Within 60 pL droplets, the average number of cells per droplet volume (λ) can be calculated for concentrations ranging from 10^2 to 10^7 CFU/mL (percentages of droplets with 0, 1 or 2 cells). The result showed that the ratio of droplets with multi-cell occupancy was statistically insignificant and could be neglected for quantifying samples with bacterial density ranging from 10^2 to 10^7 CFU/mL.

Table S1. Percentages of droplets (~60 pL) with 0, 1 or 2 cells.

Concentration of *E. coli* cells (CFU/mL)	n (= 0)	n (= 1)	n (= 2)	n (≥ 3)
10^2	0.9999	0.0000	0.0000	0.0000
10^3	0.9999	0.0001	0.0000	0.0000
10^4	0.9994	0.0006	0.0000	0.0000
10^5	0.9940	0.0060	0.0000	0.0000
10^6	0.9420	0.0565	0.0017	0.0000
10^7	0.5488	0.3293	0.0988	0.0231

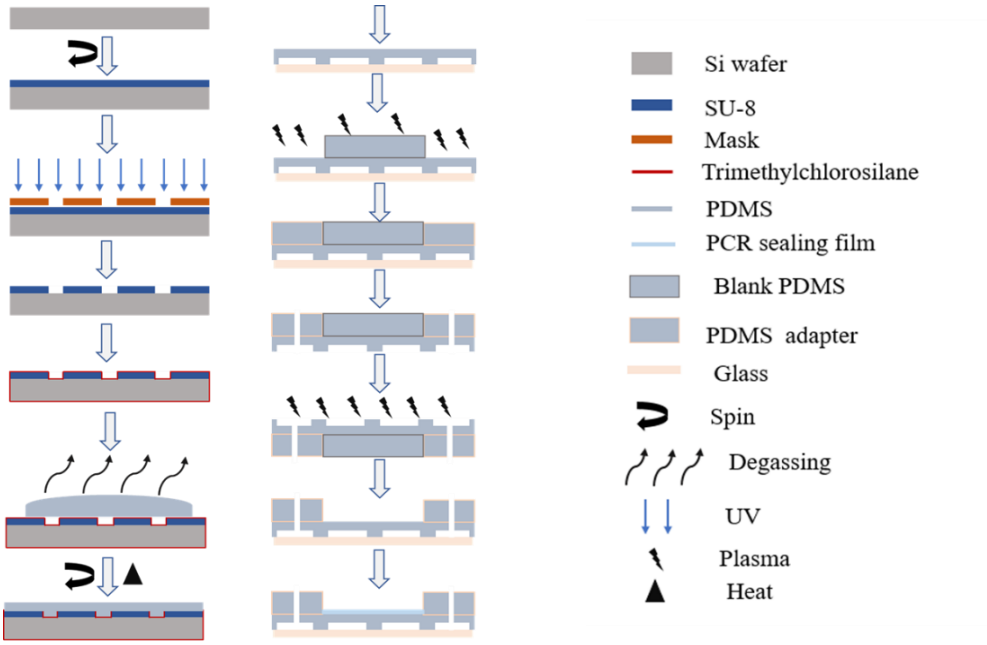

Figure S1. The process flow of chip fabrication.

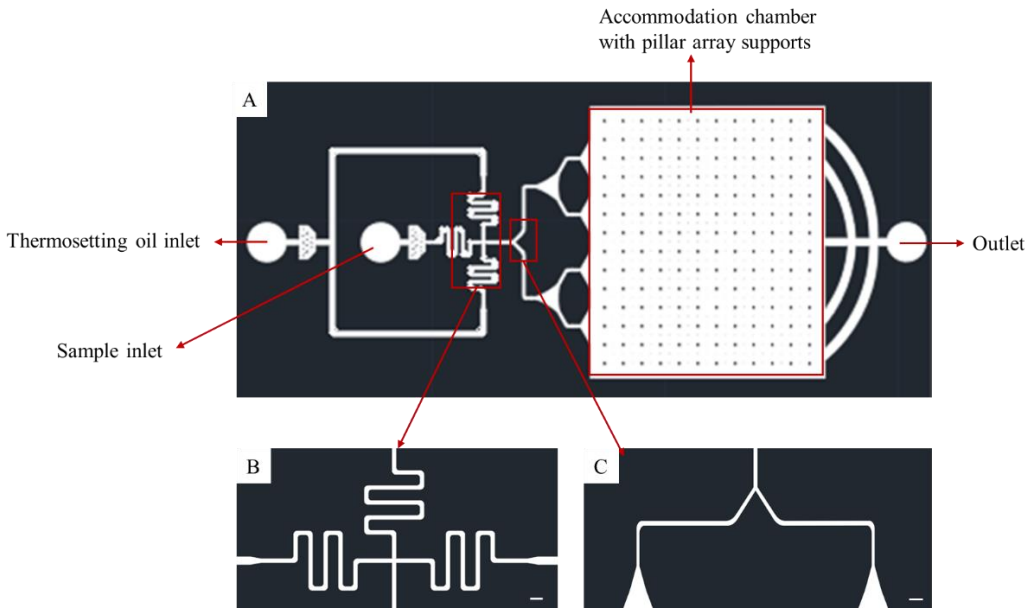

Figure S2. (A) Diagram of the chip design. This chip contains three units, including (B) flow focusing droplet generator, (C) droplet splitting region, and droplet accommodation chamber. The channel depth is 50 μm. Pillar array was designed to support the large accommodation chamber. Scale bar is 150 μm.