

## Supplementary Information

### Capillary-based Integrated Digital PCR in Picoliter Droplets

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## 1 Best annealing temperature for qPCR

We first ascertain the best performance of qPCR for LunX mRNA, by choosing a best annealing temperature. To achieve this goal, we prepare a concentration of cDNA (1uM) works at twelve annealing temperatures between 54°C-68°C as shown in Fig.S1(a), and their individual samples are placed in 12 reaction columns in one chamber. Their results are shown in Fig.S1(b), temperatures of 56°C (red line) and 57.7°C (light blue line) are the best ones in terms of annealing/extension. With temperatures higher than 63.4°C (deep blue line), data are still negative after 20 cycles.

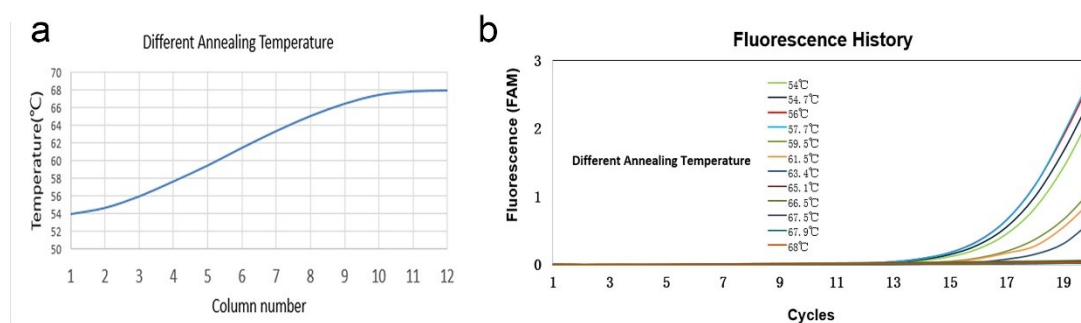


Fig.S1 Optimize the condition of qPCR experiments (a) Different annealing temperature from 54°C to 68°C; (b) Fluorescence history of different annealing temperature.

## 2 Monodisperse of droplet produced by Microtee

The homogeneous W/O droplets generated by Microtee as shown in Fig.S2a. The mean diameter of droplets from outlet is 60  $\mu\text{m}$ . The droplets have highly monodisperse because of the polydispersity index ( $\lambda$ ), which is defined as the ratio of the standard deviation to the mean of the droplet diameters, is only about 1.66%.

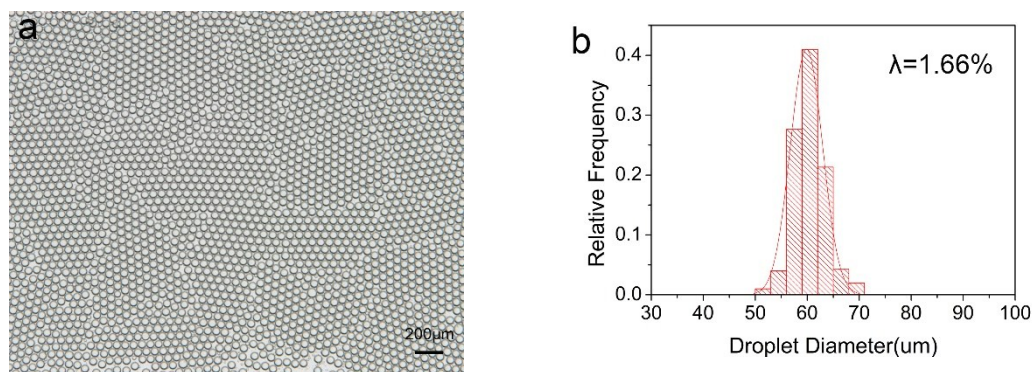


Fig.S2 Droplet generation by Microtee. (a) A monolayer of monodisperse water droplets on the glass slide. Scale bar = 200  $\mu\text{m}$ . (b) The diameter distribution of the droplets.

## 3 Numerical study of PCR thermocycler

The droplets will respond in temperature instantly to thermal changes in the carrier-fluid due to their larger surface-to-volume ratio. Moreover, the sample droplets are small in comparison

to the capillary and they can be regarded as immediate following the carrier-phase. So, this mixture fluid is simplified as a single incompressible carrier-fluid with temperature change, and the Navier-Stokes equations for the problem are as follows:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho V) = 0 \quad \text{Eq.S1}$$

$$\rho \frac{DV}{Dt} = -\nabla p + \mu \nabla^2 V + \rho g \quad \text{Eq.S2}$$

$$\rho c_p \frac{DT}{Dt} = k \nabla^2 T + \tau : \nabla V \quad \text{Eq.S3}$$

where  $D=Dt$  is the substantive derivative and represents the contraction operator of two tensors,  $\rho$  is the fluid density,  $V$  the velocity vector,  $P$  the pressure,  $\tau$  the dynamic viscosity,  $g$  the acceleration of gravity,  $C_p$  the specific heat capacity,  $T$  the temperature and  $k$  is the thermal conductivity.

In our calculation, one part of surface of capillary is contacted with the surfaces of two columns while the rest of its surface is exposed to the room air. The thermal resistance due to natural convection from the surface to air has been estimated to be between 0.1 and 1.0  $m^2k/w^{-1}$ . At the inlet, the fluid is preheated to 368 K and a constant flow rate is imposed. In addition, a no-slip boundary condition has been assumed along the walls of the channel.

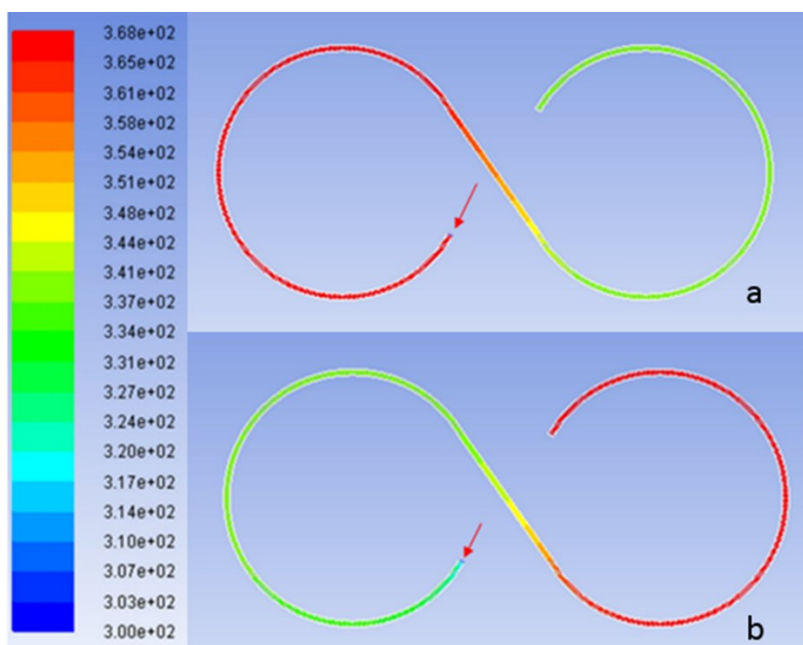


Fig.S3 Predicted temperature distribution of the oil carrier-fluid over one thermal cycles for two different inlet temperature, a  $T = 368$  k and b  $T = 338$  k. Red arrow indicates the entrance of fluid.

#### 4 Temporal number in positive droplets observed in flow cytometry

Droplets are counted temporally after amplification by flow cytometry for template concentrations from 0 to  $2.4 \times 10^4$  copies/ul. It can be seen from Fig.S4 that there are two parallel lines which stand for the two bands of 'negative' and 'positive' droplets versus time. With increasing the template concentration, the up bands are becoming dense, illustrating that the PCR works as expected.

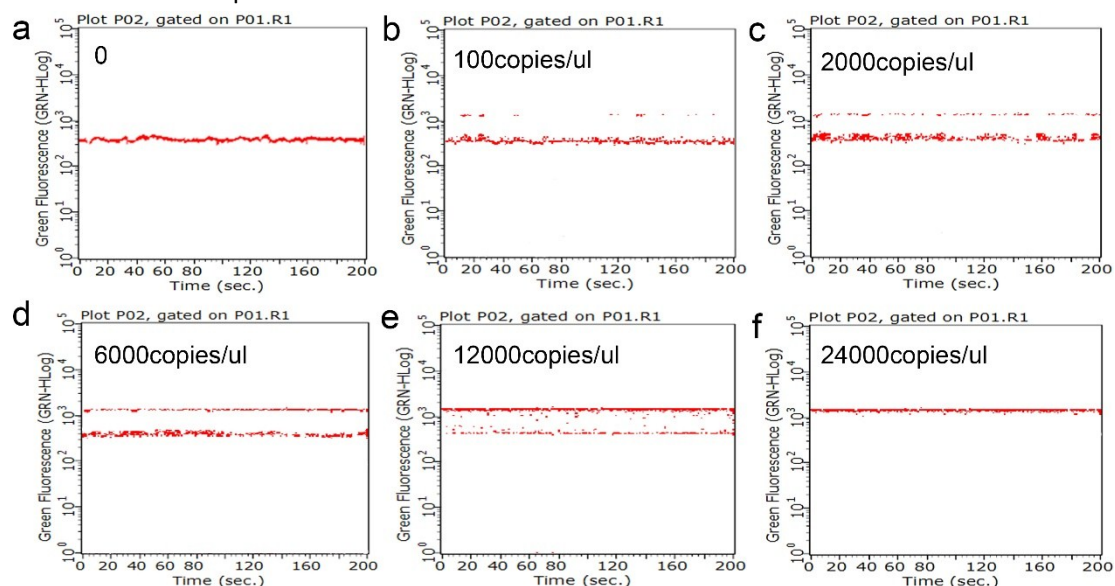


Fig.S4 On-line analysis of droplets through the integrated flow cytometry versus time with increasing the template concentration (NTC to  $2.4 \times 10^4$  copies/ul).

#### 5 On-flow ddPCR system data analysis

For the data analysis of the ddPCR system, we integrate the results of the flow cytometry with the poisson statistics. Define  $C$  as the copies of DNA template that are dispensed in the PCR mix. A volume  $V$  of PCR mix is divided into  $N$  partitions, resulting in an average volume of each partition  $\bar{v}$ . In the experiment, we can measure the positive droplets  $n$ .

$$q \stackrel{\text{def}}{=} \frac{n}{N} \quad \text{Eq.S4}$$

$$\lambda \stackrel{\text{def}}{=} \frac{C}{N} \quad \text{Eq.S5}$$

$$m \stackrel{\text{def}}{=} \frac{C}{V} = \frac{\lambda N}{V} \quad \text{Eq.S6}$$

Assuming that all the droplets are perfectly monodisperse, one has

$$N = \frac{V}{\bar{v}} \quad \text{Eq.S7}$$

Where

$$\bar{v} = \frac{4\pi r^3}{3} \quad \text{Eq.S8}$$

In this paper, we first determine  $\lambda$ , and then get the template concentration. Poisson distribution can be approximated as a binomial distribution  $n\text{-B}(\rho, N)$  when  $N$  is large enough.

The expectance and variance are

$$E(n) = N(1 - e^{-\lambda}) \quad \text{Eq.S9}$$

$$D(n) = Ne^{-\lambda}(1 - e^{-\lambda}) \quad \text{Eq.S10}$$

As for  $q = \frac{n}{N}$ ,

$$E(q) = (1 - e^{-\lambda}) \quad \text{Eq.S11}$$

$$D(q) = e^{-\lambda}(1 - e^{-\lambda})/N \quad \text{Eq.S12}$$

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

$$\rho = 1 - \left(1 - \frac{1}{N}\right)^c \approx 1 - e^{-\frac{c}{N}} = 1 - e^{-\lambda} \quad \text{Eq.S13}$$

Therefore

$$\lambda = -\ln[1 - E(q)] \quad \text{Eq.S14}$$

We use  $q$  as an unbiased estimate for  $E(q)$ , and it can be estimated as

$$\hat{\lambda} = -\ln(1 - q) \quad \text{Eq.S15}$$

In this ddPCR system, we examine  $N'$  droplets out of population  $N$ , and  $n'$  among  $N'$  droplets are found positive.

$$\frac{n}{N} = \frac{n'}{N'} \quad \text{Eq.S16}$$

$$\hat{\lambda} = -\ln\left(1 - \frac{n'}{N'}\right) \quad \text{Eq.S17}$$

Thus

$$\hat{m} = -\ln\left(1 - \frac{n'}{N'}\right)^{\frac{1}{v}} \quad \text{Eq.S18}$$

## Notes and references

1. Zhang Q, Wang W, Zhang H, et al. Temperature analysis of continuous-flow micro-PCR based on FEA[J]. Sensors and Actuators B: Chemical, 2002, 82(1): 75-81.