

An integrated microfluidic chip for nucleic acid extraction and continued cdPCR detection of pathogens

Authors:

Yaru Huang^{a,b}, ZeHang Gao^a, Cong Ma^{a,c,d}, Yimeng Sun^{a,d}, Yuhang Huang^{a,b},
Chunping Jia^{a,d*}, Jianlong Zhao^{a,d*}, Shilun Feng^{a,d*}

Affiliations:

^a Shanghai Institute of Microsystem and Information Technology, Chinese Academy of Sciences, China

^b School of Life Sciences, Shanghai Normal University, China

^c School of Information Science and Technology, ShanghaiTech University, China

^d Center of Materials Science and Optoelectronics Engineering, University of Chinese Academy of Sciences, China

***Contact:**

Shilun Feng: phone: +86-18032078750; shilun.feng@mail.sim.ac.cn

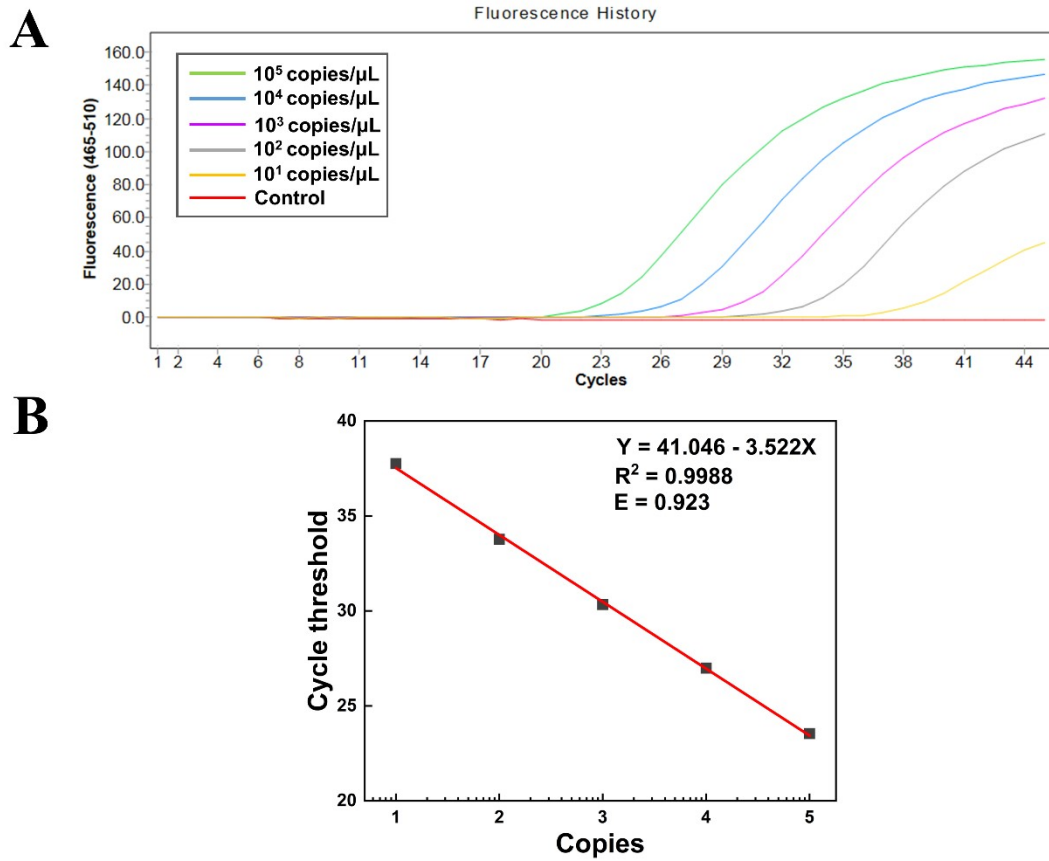


Figure S1. (A) Amplification curve of serial dilution templates for the ORF1ab gene in the qPCR system. (B) A standard curve between the log copy number of the ORF1ab gene and the reaction CT value plotted based on the results of the Fig. S1A.



Figure S2. Physical image of the experimental setup. (A) Magnet apparatus for driving bead transfer. (B) An in-situ thermal cycler instrument used to provide the temperature cycle required for digital PCR reaction. (C) Fluorescence microscope equipped with mercury lamp and CCD image sensor for capturing fluorescence images after thermal cycling.

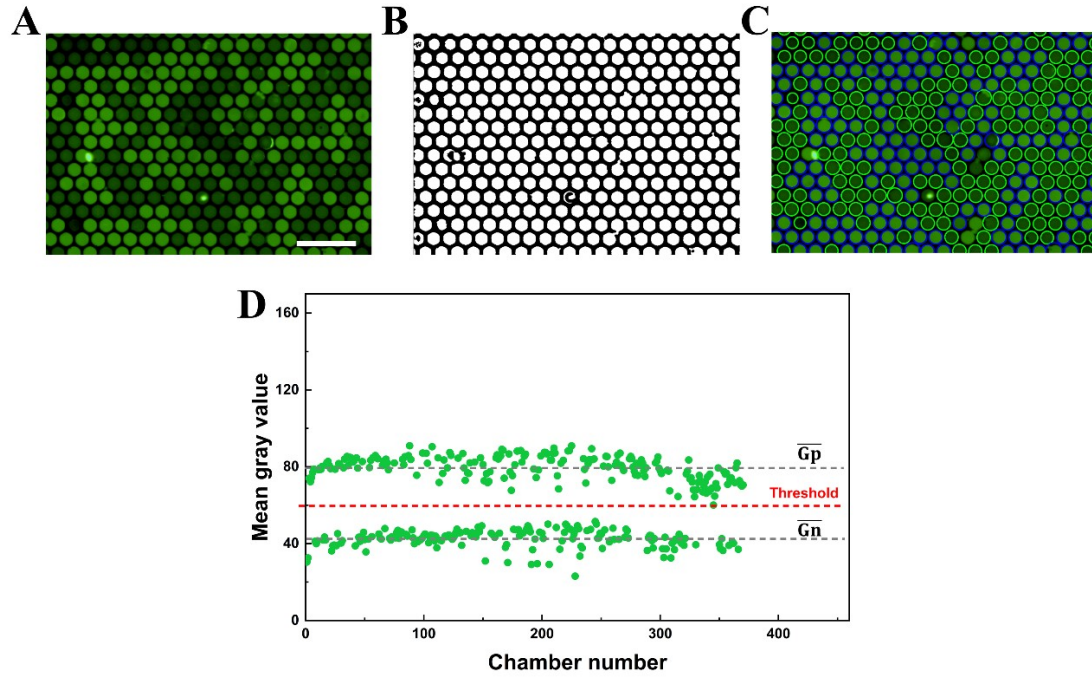


Figure S3. Digital analysis. (A) Fluorescence imaging of digital PCR results after thermal cycling, scale bar is 150 μm . (B) Signal recognition of fluorescence image: fluorescence image was binarized, and the grayscale value of each microchamber reaction unit was recognized and calculated. (C) A data analysis software (IBM SPSS Statistics, America) was used to perform the classification of microchambers with k-means clustering algorithm by their gray-scale value. (D) Statistical result of the grayscale values of all microchamber reaction units, where the average value of the negative signals was 42 and the average value of the positive signals was 79. The threshold was set according to $(\bar{G}_p + \bar{G}_n)/2$, where \bar{G}_p and \bar{G}_n corresponded to the median gray-scale value of the positive and negative chamber population. Microchambers can be easily divided into two clusters to obtain the number of positive and negative chambers.

Due to the limitations of large-field imaging, when the fluorescence results of digital PCR were taken, we usually acquired images at any three locations on the chip for statistical analysis, and estimate the total number of positive and negative microchambers of the entire chip with their average value of positive and negative signals. Ideally, when the number of partitions of digital PCR is sufficient and the partition volume is uniform, each microchamber is expected to contain 1 or 0 nucleic

acid molecule, then after digital PCR amplification, the absolute amount of template DNA in the original sample can be obtained by simply counting the number of positive microchambers. However, in actual detection, when the number of template molecules in the sample solution is large or the number of digital PCR sample partitions is not large enough, it will lead to more than one nucleic acid molecule in some partition units. In this case, if rely only on simple counting statistical methods, there will be inaccurate quantitative results. Therefore, we used the Poisson distribution to correct the quantification of nucleic acid molecules at the end of the calculation. The formula is as

follows: $C = -\ln\left(1 - \frac{d}{n}\right) / Vd$, where C represents the concentration of nucleic acid molecules in the sample solution, d represents the number of positive microchambers, n represents the total number of microchamber units in digital PCR, and Vd represents the reaction volume of each microchamber.

Based on this array, the quantitative results for serial dilution templates (5×10^4 , 10^4 , 10^3 , 10^2 , 10^1 copies/ μL) of the SARS-CoV-2 ORF1ab gene were shown as follows:

Table S1. Quantitative results for serial dilution templates of the SARS-CoV-2 ORF1ab gene

Gene	Norminal concentration (copies/ μL)	Measured concentration (copies/ μL)
ORF1ab	50000	40065.780
	10000	8918.603
	1000	1040.712
	100	79.821
	10	8.955