Supporting Information

Digital PCR Using Micropatterned Superporous Absorbent Array Chips

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Methods for microfluidic droplet digital PCR (ddPCR)

Fabrication and operation of the four-layer 4-channel microfluidic droplet generator chip was described in our previous work.[1,2] Prior to droplet generation, the glass channels were first treated with 0.1% solution of octadecyltrichlorosilane (OTS) in dry toluene for 30 min to render the channel surface hydrophobic. The carrier oil is a mixture of 39.9% (w/w) DC 5225C Formulation Aid (Dow Chemical Co., Midland, MI), 30% (w/w) DC 749 Fluid (Dow Chemical Co.), 30% (w/w) AR20 Silicone Oil (Sigma-Aldrich), and 0.1% (w/w) Triton X-100 surfactant (Sigma-Aldrich). The device was assembled with an aluminum manifold and connected with tubings for oil infusion and droplet collection. The carrier oil was injected continuously into the device by a syringe pump (Harvard Apparatus, Holliston, MA). The chip was pneumatically actuated with a constant pressure (30 kPa) and vacuum (-80 kPa) controlled by a homemade solenoid valve system to pump the aqueous solution through the channels. The pumping was conducted in a four-step fashion under the control of a program written in LABVIEW (National Instruments, Austin, TX). PCR mix contained 1x *Platinum*® *Taq* DNA polymerase buffer with 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µg/µL heat-inactivated BSA, 0.01% Tween 80, 0.4 µM each of primers for λ-DNA, 0.15 U/µL *Platinum*® *Taq* DNA Polymerase, 1X EvaGreen dye, and λ-DNA samples (0.054 and 0.54 ng/mL). Three replicates were run for each λ-DNA standard, for which 30-µL PCR mix was used to generate monodispersed droplets which were collected in 0.5 mL PCR tubes. Thermal cycling was carried out with a 2 min hot start at 95 °C, and 40 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s in a Mastercycler® nexus thermocycler (Eppendorf). Fluorescence imaging analysis was conducted following the established method to measure the percentage of positive droplets.[2, 3] ~11,000-14,500 droplets were imaged and the average droplet size was measured to be 74.8 ± 1.47 µm in diameter. The average concentration of λ-DNA standard and the expanded uncertainty were calculated following the approach described by Pinheiro, et al.[3]

Table S1. Comparison of measurement accuracy and precision of μSAAC, microfluidic droplet system and commercial platforms for dPCR analysis

dPCR system	QX100[3]	BioMark[3]	Microfluidic ddPCR	μSAAC
dPCR Format	Droplet	Microchamber array	Droplet	Microwell array
Sample loading	Automated injection	Automated injection	Automated injection	Pipetting & spontaneous filling
Partition number	~20,000	765	~11,000-14,500	2,500
DNA template	λ-DNA (NIM-001 plasmid)[a]	λ-DNA (NIM-001 plasmid)[a]	λ-DNA	λ-DNA
Relative standard uncertainty of partition volume (%)	1.0 (0.8)	4.7 (0.7)	3.4	2.9
Relative expanded uncertainty (%)	3.2 (3.6)	13 (6.0)	3.7[b]	7.0[b]

a. The values in the brackets for the commercial instruments were obtained from Ref 4 for measuring the NIM-001 plasmid reference.

b. Relative expanded uncertainty obtained for the DNA standard of 0.54 ng/mL was listed.

Reference:

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3. L. B. Pinheiro, V. A. Coleman, C. M. Hindson, J. Herrmann, B. J. Hindson, S. Bhat and K. R. Emslie, *Anal Chem*, 2012, **84**, 1003-1011.
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