Electronic Supplementary Material (ESI) for Lab on a Chip.
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A scalable self-priming fractal branching microchannel nets chip for digital PCR

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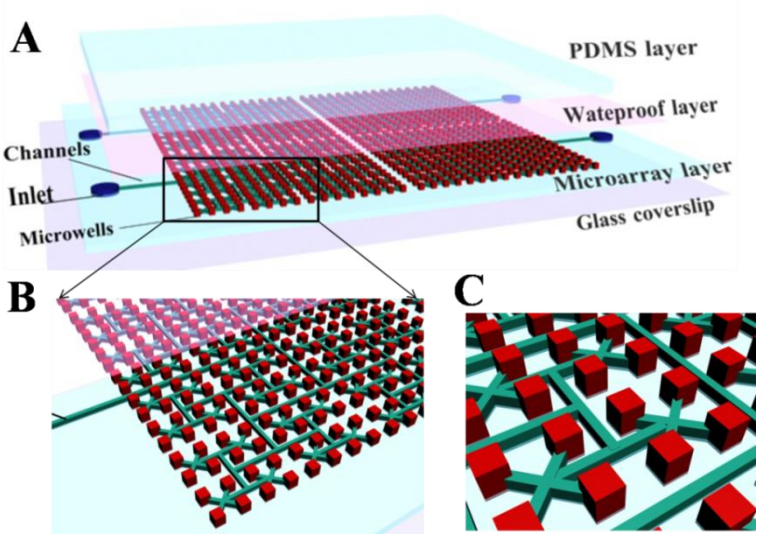

Figure S1. Schematic drawing of the fractal branching microchannel nets chip. (A) Schematic diagram of the layered structure of the chip, which is composed of two layers of polydimethylsiloxane PDMS (inlets and outlets PDMS layer and Microarray layer), a nano-waterproof layer and a glass coverslip layer. Microarray layer is composed of 4 reaction panels and each reaction panel has 4096 microwells for dPCR reaction. The whole chip has 16384 microwells in total. The nano-waterproof layer is designed to prevent water evaporation. (B) and (C) Diagram of the detail of the chip design. The main channel was divided vertically looks like a "T" into thousands of branches step by step, the green line represents the main channel and the red boxes represent the reaction chamber, the end of each branch connected with four microchambers. The volume of the reaction chamber is 150 μm ×150 μm ×250 μm, the size of the circulation channel is 50 μm× 25 μm. Photograph of the prototype device. The chip size is 40 mm × 60 mm.

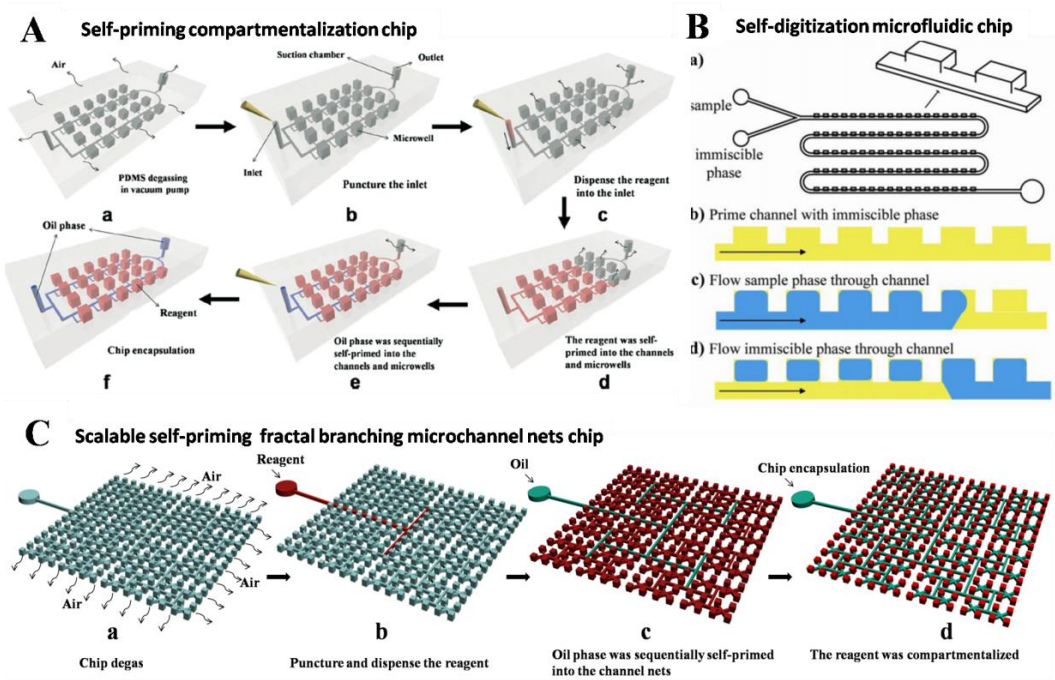

Figure S2. Design structure comparison of three kinds of digital PCR chip. (A) Self-priming compartmentalization[1]. The sample is self-priming compartmentalized by oil phase. (B) Self-digitization microfluidic chip. The sample is digitized by priming the main channel and side cavities with an immiscible phase[2]. (C) Scalable self-priming fractal branching microchannel nets chip. The main channel is divided vertically step by step into thousands of branches with a rectangular shape, the end of each branch is connected with four microwells, which makes the perfectly uniform distribution of sample and reagents. The chip has only four inlets for four reaction panels without any outlets avoiding any reagent and sample loss. Another interesting feature of the compelling design is expandability.

Figure S3. Photograph of the high-density self-priming fractal branching microfluidic nets digital PCR chip. The chip has 14 branching levels only in one fractal dimension which contains 16384 microchambers. The volume of the reaction chamber is 150 μm ×150 μm ×250 μm, the size of the circulation channel is 50 μm× 25 μm. The chip size is 40 mm ×60 mm.

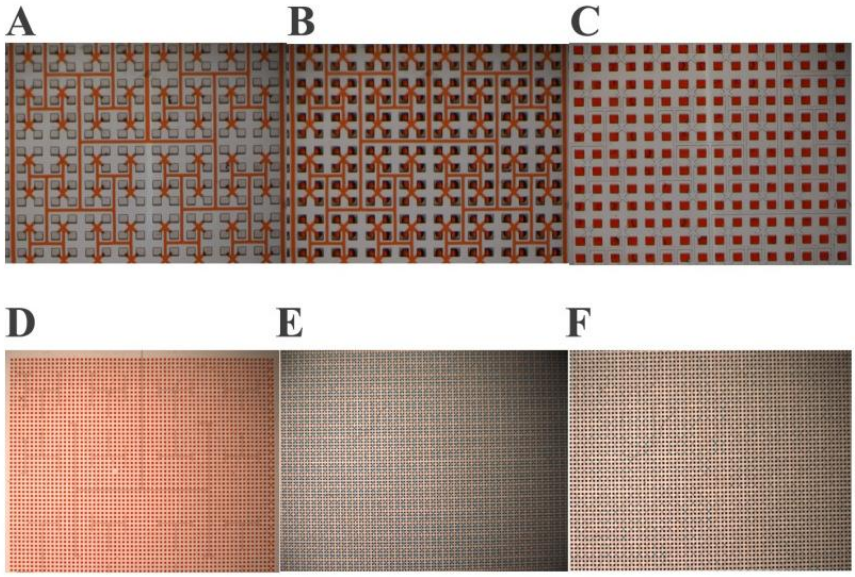

Figure S4. Sample multiple injection processes of the high-density self-priming fractal branching microfluidic nets digital PCR chip. (A) Equal distribution of 5 μL Red dye in the microchannels after injection for 5 seconds. (B) and (C) were the observation after injection for 10, 20 seconds. (D) and (E) were the second and third-time injection with 5 μL green and blue dye, respectively. (F) Every microwell are full of dye mixed well and isolated by oil phase without residue in the microchannels.

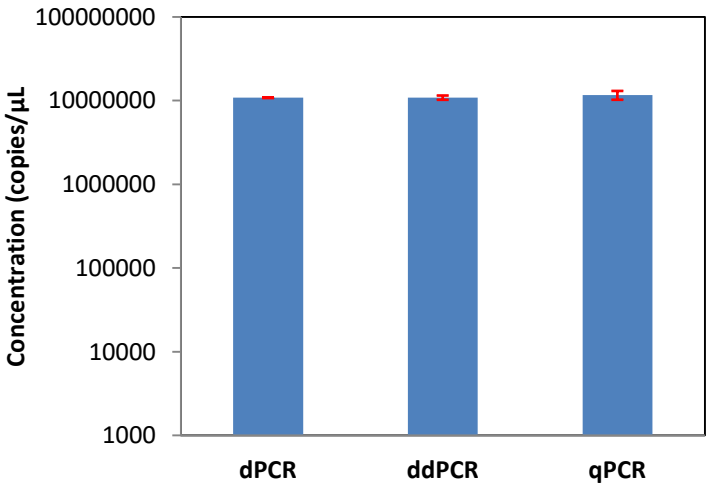

Figure S5. Comparison of self-priming fractal branching microchannel nets digital PCR, real-time PCR and bio-rad droplet digital PCR quantification results of copy number of β-actin gene.

Video S1. The operation procedure of the scalable self-priming fractal branching microfluidic nets chip. After the chip is degassed in a vacuum pump and then an adhesive tape was attached to seal the top surface of the chip. When the adhesive tape was punctured, and the red dye solution was dispensed into the inlet, the red dye was sucked into microwells quickly. When the oil was dispensed into the inlet, the oil phase was self-primed into the channels; At last, all the solution was partitioned into each microwell by the oil, no any sample was wasted.

Video S2. The procedure of multistep loading solution into the scalable self-priming fractal branching microfluidic nets chip. The chip is degassed in a vacuum pump and was sealed by an adhesive tape. When the adhesive tape was punctured, and the red dye solution and blue dye solution were dispensed into the inlet sequentially, the red dye and blue dye were sucked into microwells sequentially. There was no air bubble formed between the two liquid interfaces.

Table S1. The statistical analysis result of the digital PCR

	X_{dil}	Observed value			Average value	Average value of - ln (1-f_0)	STDEV	CV%
		1	2	3				
β-actin copy number	0.001	2587	2835	2681	2701	1.077	125.20	4.64%
	0.0001	268	277	275	273.3	0.069	4.73	1.73%
	0.00001	26	29	34	29.7	0.0073	4.04	13.62%
	0.000001	3	4	6	4.3	0.0011	1.53	35.25%

Table S2. The digital PCR statistical analysis result of the hHF-MSC stem cell marker

	X_{dil}	Observed value			Average value	Average value of - ln (1-f_0)	STDEV	CV%
		1	2	3				
ALCAM Copy number	0.1	4027	4011	3955	3997. 7	3.776	37.81	0.95%
	0.01	504	479	493	492	0.128	12.53	2.55%
	0.001	65	56	67	63	0.0154	5.86	11.27%
	0.0001	7	4	5	5.3	0.0013	1.53	24.12%

References

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2. D. E. Cohen, T. Schneider, M. Wang and D. T. Chiu, *Anal Chem*, 2010, **82**, 5707-5717.