**Supplementary Material (ESI) for Lab on a Chip**

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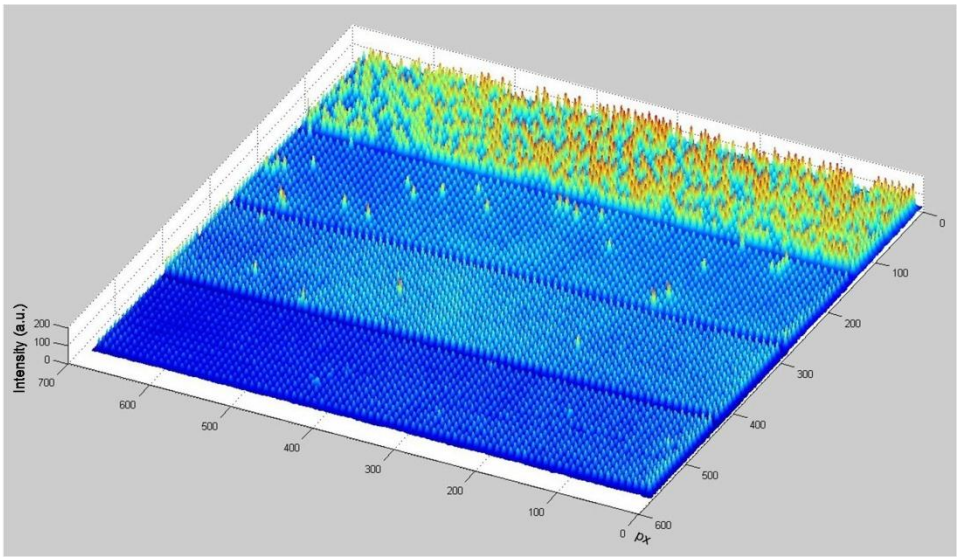


**Fig. S1** An image of fluorescence intensity taken by the MATLAB analysis. It demonstrates that the fluorescence intensity in the positive microchambers increased observably compared to the negative microchambers

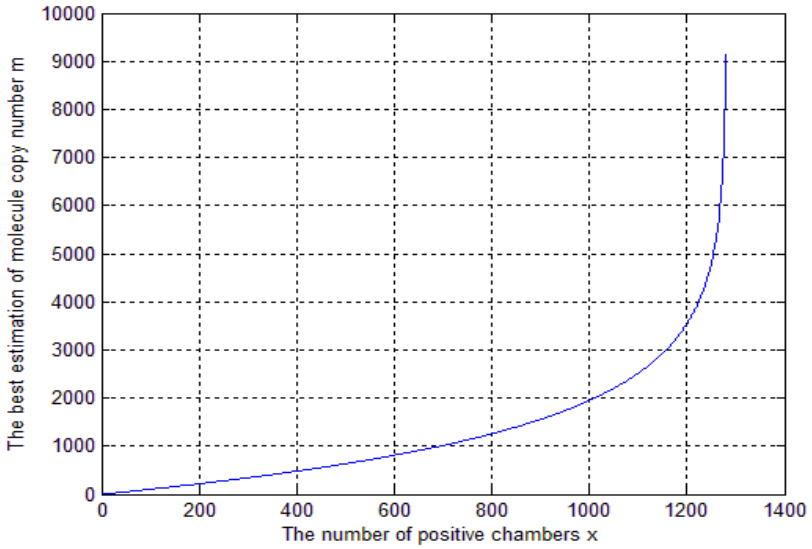


**Fig. S2:** The best estimation of molecule copy number versus the number of positive chambers. According to the curve, one can get the calculation of the true gene copy number in the panel.

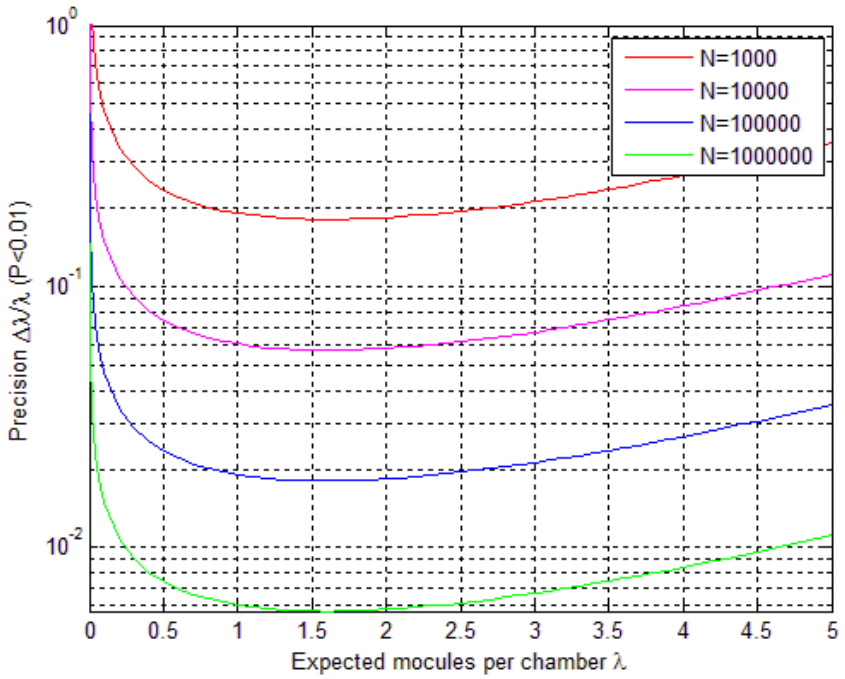


**Fig. S3:** the scaling of digital PCR precision with expected molecules per chamber.

**Fig. S4:** The air-tight packaging bottle of the self-priming compartmentalization digital PCR microchip. The lifespan of the self-priming capability was tested using an air-tight packaging bottle was used, the self-priming capability of the chip can last for one week, which is significant for practical issues. If, excellent air tightness measures were employed, the self-priming capability of the chip can last for a longer time, which will have more outstanding application merits in biological fields.

**Table S1: The statistical analysis result of the digital PCR**

| | $X_{dil}$ | Observed value | | | Average value | STDEV | Expected value | Average value of - ln (1-$f_0$) | STDEV |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | | | |
| β-actin DNA | 0.001 | 873 | 867 | 874 | 871 | 3.8 | 883 | 1.14 | 0.009 |
| | 0.0001 | 84 | 88 | 83 | 85 | 2.6 | 142 | 0.07 | 0.002 |
| | 0.00001 | 22 | 4 | 15 | 14 | 9.1 | 15 | 0.01 | 0.007 |
| | 0.000001 | 4 | 1 | 0 | 2 | 2.1 | 1 | 0.001 | 0.001 |

**Table S2: The statistical analysis result of three genes from A549 cell lines using the SPC digital PCR**

| | $X_{dil}$ | Observed value | | | Average value | STDEV | Corrected value | STDEV |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | | |
| PLAU | 0.1 | 1050 | 1015 | 1035 | 1033 | 17.56 | 3246 | 139.74 |
| | 0.01 | 146 | 141 | 158 | 148 | 8.74 | 243 | 15.23 |
| | 0.001 | 10 | 12 | 18 | 13 | 4.16 | 21 | 6.48 |
| | 0.0001 | 2 | 3 | 2 | 2 | 0.58 | 4 | 0.89 |
| ENO2 | 0.1 | 636 | 627 | 604 | 622 | 16.50 | 1312 | 49.18 |
| | 0.01 | 74 | 78 | 68 | 73 | 5.03 | 116 | 8.21 |
| | 0.001 | 12 | 13 | 15 | 13 | 1.53 | 21 | 2.38 |
| | 0.0001 | 2 | 1 | 2 | 2 | 0.58 | 3 | 0.89 |
| PLAT | 1 | 388 | 398 | 393 | 393 | 5 | 723 | 11.10 |
| | 0.1 | 31 | 28 | 35 | 31 | 3.5 | 49 | 5.54 |
| | 0.01 | 4 | 3 | 5 | 4 | 1 | 6 | 1.54 |
| | 0.001 | 1 | 1 | 2 | 1.3 | 0.58 | 2 | 0.89 |

**Table S3: Primer and probe sequences used in this study**

| Target | Description | Concentration | Sequence |
|---|---|---|---|
| β-actin DNA | Sense primer | 500nM | GGTCATCACCATTGGCAATG |
| | Anti-sense primer | 500nM | TCCATGCCCAGGAAGGAA |
| | Taqman probe | 500nM | FAM-CGGTTCCGCTGCCCTGAGGC-TAMRA |
| PLAU | Sense primer | 900nM | ABI Taqman Assay ID: Hs00938315_m1 |
| | Anti-sense primer | 900nM | |
| | Taqman probe | 250nM | |
| ENO2 | Sense primer | 900nM | ABI Taqman Assay ID: Hs01547054_m1 |
| | Anti-sense primer | 900nM | |
| | Taqman probe | 250nM | |
| PLAT | Sense primer | 900nM | ABI Taqman Assay ID: Hs00157360_m1 |
| | Anti-sense primer | 900nM | |
| | Taqman probe | 250nM | |

## Note S1: Aqueous distribution characterization in microwells

After the PDMS was degassed using the vacuum pump, the energy for driving the sample was stored in the bulk of the chip itself. During the process of sample introduction, the microwells could supply a continuous driving force until the chamber was totally full and compartmentalization fulfilled. The uniformity of microwell volumes is an important factor for the digital PCR response. In order to analyze the aqueous distribution uniformity in the SPC chambers, an image of the fluorescence of the microwells filled with calcein taken with a Maestro Ex IN-VIVO Imaging System (CRI Maestro) was used to demonstrate the uniformity of each chamber by counting the fluorescence intensity. By comparing to the fluorescent intensity of 1280 microwells measured with the average fluorescence intensity of all measured microwells, the variation of chamber volumes is about 9.4%, which demonstrated the uniformity of all chambers.

## Note S2: Dehydration of PCR reactions

The fluorosilane polymer was employed to prevent the dehydration of nanolitre volume PCR reaction. The thickness of the PDMS between the top of the chamber and the fluorosilane polymer membrane was only designed to be 100μm which is thin enough to ensure minimal evaporation of PCR reactions during thermocycling. The total volume fraction of PCR reagent in the PDMS slab is given by

$$V_{fraction} = \frac{n \times A_{chamber} \times h_{chamber}}{A_{PDMS} \times h_{PDMS}} = \frac{1}{5}$$

Where A and h refer to the area and height respectively. Assuming a saturated concentration of water vapor in PDMS at 70 oC to be 400 mol/m3, the maximum fractional loss of water from the reaction chambers is:

$$W_{loss}\% = \frac{\left(1 - V_{fraction}\right) \times C_{sat}}{V_{fraction} \times \frac{\rho_{water}}{M_{water}}} \times 100\% = 2.88\%$$

This amount of water loss does not inhibit PCR amplification and is much less than the integrated Parylene C membrane. This method of preventing from evaporation of water has the advantages of simple fabrication, lower manufacturing cost, convenient operation, etc.

## Note S3: Dynamic range

As for digital PCR, each chamber has not more than one molecule. So the dynamic range of the device can be obtained when the array becomes completely saturated. Let N denote the number of chambers in an array, m the number of DNA molecules loaded into the array, and t the number of negative chambers. We can easily obtain the probability that a DNA molecule is captured by a specific chamber is $q = \frac{1}{N}$.

Define the number of DNA molecules captured by a specific chamber as a random variable X and X meets the conditions of the binomial distribution. That is $X \sim B(m, q)$. When concentrations of the sample is quite high and the number of chamber is great, based on the theory that binomial process can be approximated by Poisson process, the number of DNA molecules a specific chamber captured may be modeled as a Poisson process with mean and variance to be $\frac{m}{N}$.

$$P(x = k) = C_m^k q^k (1 - q)^{(m-k)}$$

$$P(x = k) = \frac{\frac{m^k}{N}}{k!} e^{-\frac{m}{N}}$$

If the assumed probability of a chamber to be negative is p, when p is equal to 0 which means it captures no DNA molecule at all,

$$p = P(x = 0) = e^{-\frac{m}{N}}$$

Defined negative chambers to be a random variable Y and Y satisfies the conditions of the binomial distribution. When concentrations of the sample is so high that $m \gg N$, the occurrence of negative is a rare event and p is small. Again we use Poisson distribution to approximate binomial distribution, mean and variance is

$$\lambda = Np = N \cdot e^{-\frac{m}{N}}$$

And the probability of occurrence of t negative chambers can be calculated as follows

$$P(y = t) = \frac{\lambda^t}{t!} e^{-\lambda}$$

For $\lambda > 10$, the probability of the array being completely saturated is less than 0.01%, which can be accepted failure rate. So the maximum detectable amount m meets the inequality $N \cdot e^{-\frac{m}{N}} > 10$, which leads to the inequality $m < -N \ln \frac{10}{N}$. For an array of 1280 chambers, this is equal to 6210.

## Note S4: Digital PCR response

Random and independent distribution of target DNA molecules throughout partitions is critical to accurate digital PCR detection. Template abundance can be calculated according to the Poisson distribution formula:

$$P(n, \lambda) = \frac{\lambda^n \cdot e^{-\lambda}}{n!}$$

Where n is the number of DNA molecules per reactor and λ is the ratio between the number of DNA templates and the number of

reactors on chips. The probability of having no template inside the chamber is

$$P(n = 0, \lambda) = e^{-\lambda}$$

While the chance of having at least one copy per chamber is given by

$$P(n > 0, \lambda) = 1 - P(n = 0, \lambda) = 1 - e^{-\lambda}$$

The average number of copies per chamber for each dilution, λ, equals the product of the stock concentration c0 (molecules per chip panel volume) and the dilution factor Xdil, $\lambda = c_0 \cdot X_{dil}$ and $X_{dil} = 1$ for the stock solution, while for a 100-fold dilution of the stock solution, we have $X_{dil} = 0.01$, and so forth. Each chamber containing one or more templates should produce a positive signal; therefore, the observed fraction of positive chambers, $f_0$ should equal to $P(n > 0, \lambda)$ on the chip. We have $f_0 = 1 - e^{-c_0 \cdot X_{dil}}$, which can be rewritten as

$$\ln(1 - f_0) = -c_0 \cdot X_{dil}.$$

A curve is made to relate the fraction of the positive chambers ($f_0$) to the dilution factor $X_{dil}$. It was found a linear variation of the fraction of the positive chambers with different DNA template concentrations. Then, a regression curve was acquired by plotting the $-\ln(1 - f_0)$ against the dilution factor $X_{dil}$, which is the linear form of the Poisson distribution equation. The initial concentration of the DNA stock solution from the linear regression fit was determined.

The result demonstrated that our chip could produce robust result following the Poisson distribution law. Therefore, the chip could be used to calculate the absolute DNA concentration precisely.

## Note S5: Determining DNA Copy Number

When single DNA molecules are randomly partitioned into these chambers, it is possible that two or more molecules are present in the same chamber. The probability increases as the number of molecules per panel (or DNA concentration) increases. As a result there may be more molecules in each panel than there are calls. Therefore, a mathematical correction is performed. The calculation is derived from the total number of chambers in the panel and the number of detected calls.

Each panel in a digital PCR chip contains 1280 separate reaction chambers. The number of chambers is sufficiently large to use Poisson probabilistic analysis to broadly estimate the mean concentration of the DNA sample using the following formula:

$$\left( ln\left( \frac{1280 - number\ of\ positive\ chambers}{1280} \right) \right) \cdot (-1280)$$

A digital PCR panel can be considered a random statistical sampling of a large universe of chambers containing DNA molecules, and the mathematical correction permits 95% confidence interval estimation background.

## Note S6: Derivation of measurement uncertainty equation

The number of template molecules per panel, m, following analysis of a diluted solution of DNA molecules was estimated using binomial approximation based on the number of chambers containing amplified product, H, and the total number of chambers analyzed, N. The measurement uncertainty for m, $u_m$, was derived as follows: the probability that a single partition will be positive, p, can be estimated as $\frac{H}{N}$ with a standard deviation of $\sqrt{\frac{P(1-P)}{N}}$, As the number of chambers in a panel becomes large, the true number of target molecules per chamber, λ , is

$$\lambda = -\ln(1 - P)$$

Following this equation:

$$\frac{\partial \lambda}{\partial P} = \frac{-1}{1 - P} \times \frac{\partial (1 - P)}{\partial P} = \frac{1}{1 - P}$$

Therefore, the standard uncertainty of λ, $u_\lambda$, is estimated as:

$$u_\lambda = \frac{1}{1 - P} \times u_P = \sqrt{\frac{P}{N(1 - P)}}$$

Since m=λN, the standard uncertainty for m can be estimated as:

$$u_m = \sqrt{\frac{NP}{1-P}} = \sqrt{\frac{NH}{N-H}}$$

The relative uncertainty for m is:

$$\frac{u_m}{m} = \frac{1}{-\ln(1-\frac{H}{N})}\sqrt{\frac{H}{N(N-H)}}$$

The uncertainty for m is thus related to both the number of chambers analyzed and the number of positive chambers. Utilizing this mathematical analysis, the optimal number of positive chambers needed to obtain tightest confidence interval was presented. This can provide computation of maximal resolution of copy number variation using a self-priming compartmentalization digital PCR chip.