## Supplementary material

## High-Precision Digital Droplet Pipetting Enabled by Plug-and-Play

# **Microfluidic Pipetting Chip**

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### Experimental setup of the digital droplet pipette

The digital droplet pipetting device is composed of four parts: the program control unit, the microfluidic pipetting chip, the electromagnetic actuator and the exterior plastic case amounted onto the common micropipette (Dragon-LAB TopPette). The exterior plastic case was designed in 3D modeling software and fabricated by acrylic sheets. The customized control unit provides programmable pulsed wave to drive electromagnetic actuator (10ms width, 100ms period, and 5V amplitude). The printing actuator was show in Fig. 1b, a stainless steel beam (30mm length and 5mm width) with a tin pin (Keystone Electronics 1359-1) attached adheres to an electromagnetic relay (Omron G2R-1A-E-5VDC). A miniature linear stage (NEWPORT M-MR1.4) was used to adjust the distance between the pin and the microfluidic pipetting chip.

Experimental schematic of the droplet size calibration.



Photron FASTCAM SA5 High-Speed Camera

Figure S1. Schematic illustration of the droplet size calibration by using a stroboscopic.



Performance of dispensing protein solutions

**Figure S2.** a) Image of individual BSA droplets in the oil. Scale bar: 200µm. b) Probability distribution histograms of droplet diameters.

An experiment to evaluate the performance of dispensing protein solutions has been conducted. Bovine serum albumin solution (BSA, 20µg/mL) was used as a sample reagent. In the experiment, 150 BSA droplets were dispensed in a petri dish containing an oil layer by the droplet pipette. The Fig. S2a shows a microscopic image (Leica DMI3000b) of droplets, the diameter distribution histogram by ImageJ from the dispensed droplets have been shown in Fig. S2b, which the diameter CV of droplets is 2.4%.

#### Error analysis of digital droplet pipette

The diameter of a single dispensed droplet is D, which follows a normal distribution  $N(D, \sigma^2)$ . According to the propagation of error formula,<sup>1</sup> the random errors (expressed by coefficient of variation) of droplet volume is :

$$CV_{volume} = \frac{\sigma_V}{V} = \frac{\sqrt{\left(\frac{\partial V}{\partial D}\right)^2 \sigma^2}}{V} = 3\frac{\sigma}{D} = 3CV_{diameter}.$$
 (Eq. S1)

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$$CV_{dispensed} = \frac{\sqrt{n\sigma_V}}{V_a} = \frac{\sigma_V}{\sqrt{\nu}V} = \frac{1}{\sqrt{n}}CV_{volume} = \frac{3}{\sqrt{n}}CV_{diameter}.$$
(Eq. S2)

According to the error analysis of pipette,<sup>2</sup> the imprecision and inaccuracy of dispensed volume (V) is  $\sigma$  and u. The total random error (CV) is:

$$CV = \sqrt{\sigma^2 + u^2}$$
(Eq. S3)

#### Theoretical error analysis of dilution concentration

Concentration diluted by two diluting methods was conducted by using digital droplet pipette and standard micropipette, the theoretical errors of the concentration is calculated by using the theory of error synthesis and the calculation process is as described below. The relevant data of digital droplet pipette were measured by imaging and gravimetric characterizations which were mentioned above. And the data of micropipettes were got from Finnpipette®F1 User Manual.<sup>3</sup>

#### Part 1: Dilution using digital droplet pipette

The volume of a single dispensed droplet is  $V_d$ , which follows a normal distribution  $N(V_d, \sigma_d^2)V_a = \sum_{i=1}^n V_d$ . The number of dispensed sample droplets is n, and the total volume of sample in one well is follows  $N(nV_d, n\sigma_d^2)$ .

The volume of diluent (dispensed by micropipette, Finnpipette®F1, volume range are  $20 - 200 \mu L$ ) in one well is  $V_b$ , the imprecision is  $\sigma_b$ , the inaccuracy is  $u_b$ . The concentration of sample in this well is:

$$C = \frac{nV_d}{nV_d + V_b} C_0 = \frac{V_a}{V_a + V_b} C_0$$

Where  $C_0$  is a constant value of sample stock concentration. According to the propagation of error formula, the imprecision of C is:

$$\sigma_{c} = \sqrt{\left(\frac{\partial C}{\partial V_{a}}\right)^{2} \sigma_{a}^{2} + \left(\frac{\partial C}{\partial V_{b}}\right)^{2} \sigma_{b}^{2}} = \sqrt{\left(\frac{V_{b}C_{0}}{(V_{a} + V_{b})^{2}}\right)^{2} \sigma_{a}^{2} + \left(\frac{V_{a}C_{0}}{(V_{a} + V_{b})^{2}}\right)^{2} \sigma_{b}^{2}} = C_{0} \frac{\sqrt{V_{b}^{2} \sigma_{a}^{2} + V_{a}^{2} \sigma_{b}^{2}}}{(V_{a} + V_{b})^{2}}$$

The inaccuracy of *C* is:

$$u_c = \sqrt{\left(\frac{\partial C}{\partial V_b}\right)^2 {u_b}^2} = C_0 \frac{V_a u_b}{\left(V_a + V_b\right)^2}.$$

The total random error of C is: (the number of repeat measurement is s = 6)

$$\sigma = \sqrt{\frac{1}{s}\sigma_c^2 + u_c^2} = \frac{C_0}{(V_a + V_b)^2} \sqrt{\frac{V_b^2 \sigma_a^2 + V_a^2 \sigma_b^2}{s} + V_a^2 u_b^2}$$

Thus, we can estimate the coefficient of determination (CV) of C to be:

$$\frac{\sigma}{C} = \frac{1}{(V_a + V_b)V_a} \sqrt{\frac{V_b^2 \sigma_a^2 + V_a^2 \sigma_b^2}{s} + V_a^2 u_b^2}$$

$$=\frac{1}{(nV_d+V_b)V_d}\sqrt{\frac{V_b^2\sigma_d^2}{ns} + \frac{V_d^2\sigma_b^2}{s} + V_d^2u_b^2}$$
 (Eq. S4)

The CV of C decreases with the increasing of n.

## Part 2: Dilution using common pipetting

Dilution with micropipette requires two iterations. The primary sample was firstly diluted 20 times, diluted sample concentration is:

$$C' = \frac{V_0 C_0}{V_0 + V_1} = \frac{C_0}{20}$$

where  $V_0 = 20\mu L$  and  $V_1 = 380\mu L$  are the dispensed volume of sample and diluent using two micropipettes (Finnpipette®F1, volume range are  $2 - 20\mu L$  and  $100 - 1000\mu L$ ). The imprecision of  $V_0$  and  $V_1$  are  $\sigma_0$  and  $\sigma_1$ , where  $\sigma_0 = 0.1\mu L$ ,  $\sigma_1 = 3\mu L$ . The inaccuracy of  $V_0$  and  $V_1$  are  $u_0$  and  $u_1$ , where  $u_0 = 0.2\mu L$ ,  $u_1 = 8\mu L$ . Similarly, using the propagation of error formula, the imprecision of C is:

$$\sigma' = C_0 \sqrt{\frac{V_1^2 \sigma_0^2 + V_0^2 \sigma_1^2}{(V_0 + V_1)^4}} = 0.00044C_0$$

The inaccuracy of C' is:

$$u' = C_0 \sqrt{\frac{V_1^2 u_0^2 + V_0^2 u_1^2}{(V_0 + V_1)^4}} = 0.00111C_0$$

In the 2nd iteration, the volume of diluted sample in this well is  $V_A$ , the volume of the diluent is  $V_B$ . The concentration of sample in this well is:

$$C'' = \frac{V_A C}{V_A + V_B}.$$

Using the same method as mentioned above, the total random error of concentration of  $C^{"}$  is: (the number of repeat measurement is s = 6)

$$\sigma = \frac{C_0}{20(V_A + V_B)^2} \sqrt{\frac{V_B^2 \sigma_A^2 + V_A^2 \sigma_B^2 + 0.176V_A^2(V_A + V_B)^2}{s} + V_B^2 u_A^2 + V_A^2 u_B^2 + 0.444V_A^2(V_A + V_B)^2}}{s}$$

The coefficient of variation (CV) of C'' is:

$$\frac{\sigma}{\bar{c}^{"}} = \frac{1}{(V_A + V_B)V_A} \sqrt{\frac{V_B^2 \sigma_A^2 + V_A^2 \sigma_B^2 + 0.176V_A^2(V_A + V_B)^2}{s} + V_B^2 u_A^2 + V_A^2 u_B^2 + 0.444V_A^2(V_A + V_B)^2}, \quad (Eq.$$

S5)

Where  $\sigma_A$  and  $\sigma_B$  are the imprecision of  $V_A$  and  $V_B$ ,  $u_A$  and  $u_B$  are the inaccuracy of  $V_A$  and  $V_B$ .

Concentration (μg/mL)	Theoretical errors (%) <sup>a</sup>		Measured errors (%)	
	Droplet pipetting	Common pipetting	Droplet pipetting	Common pipetting
1	1.75	40.84	3.77	49.04
2	1.69	20.52	2.37	42.00
3	1.66	13.81	2.76	37.96
4	1.65	10.50	3.34	19.94

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5	1.65	8.54	3.55	16.29
6	1.64	7.27	3.60	14.10
7	1.64	6.38	3.61	12.03
8	1.63	5.73	3.46	8.14
9	1.63	5.24	3.37	6.06
10	1.63	4.86	3.17	5.30

Table S1 The theoretical errors and measured errors caused by Droplet pipetting and Common pipetting respectively

<sup>a</sup> The calculation was based on the **Eq.S4** and **Eq.S5**.

## Reference

1. H. W. Coleman and W. G. Steele, *Experimentation, Validation, and Uncertainty Analysis for Engineers*, John Wiley & Sons, Hoboken, 4<sup>th</sup> Edition, 2009.

2. A. J. Hedges, Int. J. Food Microbiol., 2002, 76, 207-214.

3. Finnpipette®F1-Instruction for use, <u>https://assets.thermofisher.com/TFSAssets/LCD/Instructions/</u> <u>Finnpipette-F1-Instruction-For-Use-1508980-02-EN.pdf</u>