Electronic Supplementary Information (ESI)

Rapid and Multiplex Detection of Legionella's RNA using Digital Microfluidics

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Content:

Evaluation of the error caused by droplet volume variability during an exponential dilution series in digital microfluidics

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We evaluate here how the random variability in the droplet volume in digital microfluidics gives rise to an error in the reagent concentration during an exponential dilution series. To create this dilution series a buffer droplet is mixed with a reagent droplet. The resulting droplet is then split in two droplets and one of the resulting droplets is kept for the next dilution step. This process is repeated for n steps to create the exponential dilution series.

1. First dilution step

To create the first dilution step of the series, a droplet of volume V_0 and regent concentration of C_0 is mixed with a buffer droplet of volume V_B and concentration $C = 0$. The concentration C_1 of the mixed droplet is thus given by:

$$C_1 = \frac{C_0 V_0}{V_0 + V_B}$$

The relative error $\Delta C_1 / C_1$ on C_1 is thus given by:

$$\left(\frac{\Delta C_1}{C_1}\right)^2 = \left(\frac{\Delta C_0}{C_0}\right)^2 + \left(\frac{\Delta V_0}{V_0}\right)^2 + \left(\frac{\Delta(V_0 + V_B)}{V_0 + V_B}\right)^2$$

where ΔC_0 is the standard deviation of the concentration from the bulk solution, and ΔV_0 and ΔV_B are respectively the standard deviation of the volume of the reagent and buffer droplets. As both the buffer and the buffer droplets were obtained from the same on-chip dispensing protocol, we can assume that $\Delta V_0 = \Delta V_B = \Delta V$, where ΔV is the standard deviation of droplet volume following dispensing from a reservoir. We thus have:

$$\Delta(V_0 + V_B) = \sqrt{(\Delta V_0)^2 + (\Delta V_B)^2} = \sqrt{2}\,\Delta V$$

As both droplets were obtained by the same dispensing process, we also neglect herein any systematic volume difference between V_0 and V_B such that $V_0 \cong V_B = V$. We thus have:

$$\left(\frac{\Delta C_1}{C_1}\right)^2 = \left(\frac{\Delta C_0}{C_0}\right)^2 + \left(\frac{\Delta V}{V}\right)^2 + \left(\frac{\sqrt{2}\,\Delta V}{2V}\right)^2$$

$$\left(\frac{\Delta C_1}{C_1}\right)^2 = \left(\frac{\Delta C_0}{C_0}\right)^2 + \frac{3}{2}\left(\frac{\Delta V}{V}\right)^2$$

Knowing the standard deviation of the droplet volume, this expression can be used to evaluate the error on the concentration of the first dilution level.

2. Second dilution step

For the second dilution step, we first have to split the mixed droplet into two individual droplets. Neglecting systematic error that might occur during this splitting process, the volume of the new split droplet is given by:

$$V_1 = \frac{V_0 + V_B}{2}$$

Thus the error on V_1 is:

$$\Delta V_1 = \sqrt{(\Delta V_0)^2 + (\Delta V_B)^2} = \sqrt{2}\,\Delta V$$

The concentration C_2 of the mixed droplet after the second dilution step is given by:

$$C_2 = \frac{C_1 V_1}{V_1 + V_B}$$

The error on the concentration after the second dilutions step can thus be found using the same process as for the first dilution step:

$$\left(\frac{\Delta C_2}{C_2}\right)^2 = \left(\frac{\Delta C_1}{C_1}\right)^2 + \left(\frac{\Delta V_1}{V_1}\right)^2 + \left(\frac{\Delta(V_1 + V_B)}{V_1 + V_B}\right)^2$$

$$\left(\frac{\Delta C_2}{C_2}\right)^2 = \left(\frac{\Delta C_1}{C_1}\right)^2 + 2\left(\frac{\Delta V}{V}\right)^2 + \left(\frac{\sqrt{3}\Delta V}{2V}\right)^2$$

$$\left(\frac{\Delta C_2}{C_2}\right)^2 = \left(\frac{\Delta C_1}{C_1}\right)^2 + \frac{11}{4}\left(\frac{\Delta V}{V}\right)^2$$

3. n$^{\text{th}}$ dilution step

In general, it is possible to show that, for the n$^{\text{th}}$ dilution step, the error on the concentration is given by (for n>0):

$$\left(\frac{\Delta C_n}{C_n}\right)^2 = \left(\frac{\Delta C_{n-1}}{C_{n-1}}\right)^2 + \frac{5n+1}{4}\left(\frac{\Delta V}{V}\right)^2$$

This formula can be used to find the error of the n$^{\text{th}}$ dilution step knowing the error on the (n-1) step.

Using arithmetic series, we can then show that the error of the n$^{\text{th}}$ dilution step can be obtained directly from:

$$\left(\frac{\Delta C_n}{C_n}\right)^2 = \left(\frac{\Delta C_0}{C_0}\right)^2 + \frac{5n^2 + 7n}{8}\left(\frac{\Delta V}{V}\right)^2$$

Thus, if we consider that the initial concentration of the bulk solution at the beginning of the dilution series is known (i.e., $\Delta C_0 = 0$), the error on the concentration of the nth step is function of only the error on the droplet volume:

$$\frac{\Delta C_n}{C_n} = \frac{\Delta V}{V}\sqrt{\frac{5n^2 + 7n}{8}}$$

The following table provides numerical analysis of the error as a function of the dilution step:

Dilution Step	$\Delta C_n / C_n$	C_n / C_0
0	0	1
1	1.22 $\Delta V/V$	½
2	2.06 $\Delta V/V$	¼
3	2.87 $\Delta V/V$	1/8
4	3.67 $\Delta V/V$	1/16
5	4.47 $\Delta V/V$	…
6	5.27 $\Delta V/V$	
7	6.06 $\Delta V/V$	
8	6.86 $\Delta V/V$	
9	7.65 $\Delta V/V$	
10	8.44 $\Delta V/V$	
11	9.23 $\Delta V/V$	…
12	10.0 $\Delta V/V$	1/4096
13	10.8 $\Delta V/V$	1/8192

For example, assuming an initial standard deviation of $\Delta V/V = 3\%$, the standard deviation of the concentration after 13 dilutions step is of about 32%.

<u>Note:</u>
It is important to note that we considered only the random variability in droplet volume in our analysis. Systematic error would have to be taken into account separately. For example, if the buffer droplets are systematically larger than the reagent droplets or if the splitting process is systematically biased, the average concentration of the various steps of the dilution series has to be shifted accordingly