Syringe-pump-induced fluctuation in all-aqueous microfluidic system – implications for flow rate accuracy

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Supplementary information

1. Methods of measurement of frequency of ripples

Depending on the quality of videos obtained in experiments, including level of noise, contrast, and brightness, we have three methods to measure the frequency of ripples accordingly. In the following, we illustrate each method with an example, and at last, we apply the three methods to one same set of experimental images, showing that these three methods are equivalent.

a. Tracking the position of the interface

When the displacement of the interface is sufficiently large, namely more than 4 pixels, we analyze the displacement of interface as a function of time to measure the frequency of the ripples.

Starting from the experimental picture, we process the image to extract the position of the interface, as shown in Fig. S1.b, and measure the vertical position of the interface at a given location. Then we apply the same process to each frame and obtain the displacement of the interface as a function of time, as shown in Fig. S1.c. Next we apply a Discrete Fourier Transform to the oscillation of displacement and it leads to the frequency spectrum, which typically exhibits a single largest peak corresponding to the concentrated frequency, as illustrated by the black line with scatters in Fig. S1.d. Finally we assume the frequency spectrum can be fitted by a Gaussian distribution, as shown by the red continuous line in Fig S1.d. Such a fit leads to the mean value of the frequency associated to the ripples and the variance which gives us an estimation of the uncertainties.



Figure S1: Tracking the position of the interface. (a)Example of an original photograph obtained experimentally using microscope and high speed camera. (Scale bar 100 μ m.) (b) Edge-detection interface via image processing, which mainly consists of open operation, contrast enhancement, and edge detection. (c) Extracted vertical position of the point on the interface marked with red circle in Fig. S1.b as a function of time. (d) Frequency spectrum obtained via Discrete Fourier Transform (black line with squares), and Gaussian fit (red continuous line). The inset is a magnified centered view around the maximum of the Gaussian fit. The obtained frequency is (373.1 \pm 7.6) Hz, which is same as the estimated *f*_{pulse} of 376.2 Hz within experimental error.

b. Analysis of interface grayscale value

In some situations, the displacement of the interface estimated from the images using the previous method is not sufficiently accurate to allow a good analyzing, typically when displacement is smaller than 4 pixels (see Fig. S2.a), we instead observe the ripples in the middle of the jet using a measurement of the grayscale intensity.

First, we extract the grayscale intensity in each frame at a given point where ripples pass by and

thus obtain the grayscale value as a function of time (see Fig. S2.b). Then we apply a Discrete Fourier Transform and Gaussian fitting method as above, as shown in Fig. S2.c and Fig. S2.d.



Figure S2: Analysis of gray scale. (a) Original photograph obtained in experiments with microscope and high speed camera. (Scale bar 100 μ m.) (b) Extracted grayscale values changing over time of the fixed point marked with red circle in Fig. S2.a. (c)Frequency spectrum obtained via Discrete Fourier Transform (black line with squares), and Gaussian fit (red continuous line). The inset is a magnified centered view around the maximum of the Gaussian fit. The obtained frequency is (319.3±9.0) Hz, which is same as the estimated f_{pulse} of 322.5 Hz within experimental error.

c. Manually counting

When using Cole Parmer pump (Model EW-74900-45), there is another corrugation (see Fig. S3), which has a smaller frequency, typically tens of Hz, outside of the small ripples we have

observed. We think this corrugation is due to a lack of lubrication between the mechanical components in the pump as it was purchased six years ago. The corrugation makes the ripples not exactly periodic and the method presented above doesn't lead to a concentrated frequency. In this case, we count the ripples manually. We count the ripples that pass by in 0.1 s, for a few times, typically 3 times, and calculate mean frequency and the error on the assumption that the error obeys *t*-distribution. Define X_i as the data of *i*th time of counting, the mean frequency with error is calculated by

$$X = \overline{X} \pm t \, s \, / \sqrt{n}$$

where

$$\overline{X} = \frac{1}{n} \sum_{i=1}^{n} X_i ,$$

$$s = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \overline{X})^2}{n-1}}$$

n is the counting times, and *t* is the *t*-value of *t*-distribution when sample size is n.

In an example, we count the ripples for 3 times, as shown in Table S1. We choose *t*-value at 95% confidence, which is 2.35 here, and obtain the frequency with error as

$$f_{ripples} = (316.7 \pm 7.8)$$
Hz.

 Table S1: Measured data using the method of counting

	X ₁	X ₂	X ₃	X	S
Frequency/Hz	320	310	320	316.7	5.8



Figure S3: An example of the ripples observed with a larger corrugation outside. The red continuous lines are the sketched corrugation. Scale bar is $200 \ \mu m$.

We apply these three methods to a video which is suitable to each of them (see Fig. S4), whose estimated f_{pulse} in this situation is 376.2 Hz. The results are (unit: Hz)

Method a:
$$f_{ripples}$$
=371.4±12.9;
Method b: $f_{ripples}$ =371.9±11.2;
Method c: $f_{ripples}$ =372.3±4.2.

Both of them are same as f_{pulse} within experimental error, which has demonstrated that the three methods lead to the same result.



Figure S4: A set experimental images which can be applied with each of the three methods. Qin=7 mL/h. Qout=8 mL/h. Scale bar is 100 μm.

2. Same ripples observed with another syringe pump of same model (Longer Pump Model LSP01-2A)

We do the experiment with another two pumps of the same model, and have obtained the similar observation, as shown in Fig. S5.



Figure S5: Optical microscope image showing the ripples when using another Longer Pump (Model LSP01-2A). Qin=3mL/h, and Qout=6 mL/h. Scale bar is 100 μm.