

Supplementary information

1. Device fabrication

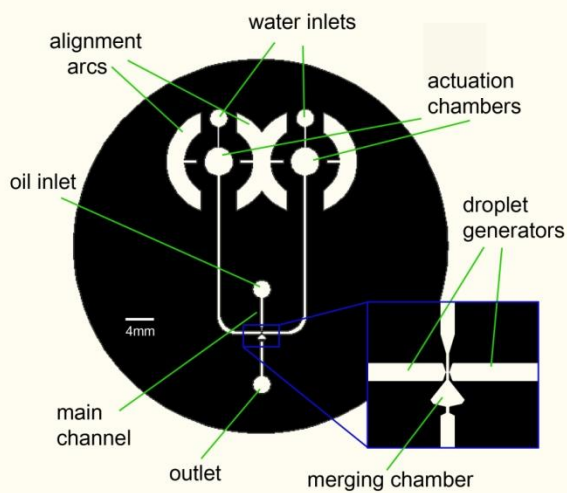


Fig. S1: transparency of the microfluidic device showing its main components.

Fig. S1 describes in detail the features of the mask used to fabricate the device. The piezoelectric actuation and accuracy were discussed in previous works^{1,2}.

2. Volume calculation and error estimation

a. First approximation

Our image analysis program measures the projected area of the droplet. The area of each droplet is measured 5-20 times over its trajectory. The most basic model to compute a droplet's volume is to assume it has a cylindrical shape and its volume is given by $V = Ah$, where V is the droplet's volume, A and h are the droplet's area and the channel depth, respectively. To measure the channel depth h , we applied a method described by J. Xu and D. Attinger³ using a custom-made guillotine to perpendicularly slice a thin cross section of the merging chamber (Fig. S2).

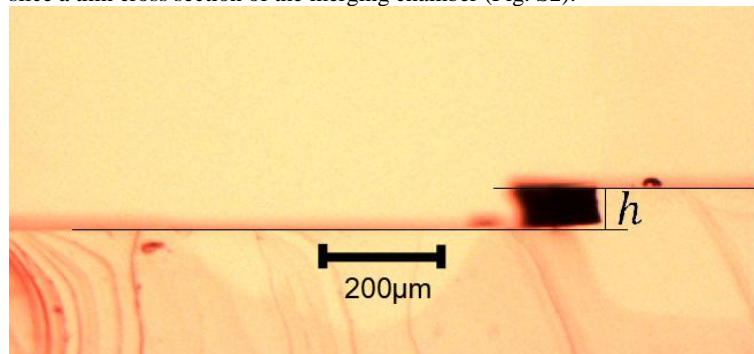


Fig. S2: Cross section of the merging chamber, sliced using a custom-made guillotine.

The channel depth was measured based on a set of three images, such as in Fig. S2. Within 80% of the central area of the merging chamber $h = 67 \mu\text{m} \pm 0.9 \mu\text{m}$. As can be seen from the figure, due to fabrication defects, deviations as high as $\pm 6 \mu\text{m}$ were measured near the edges. This demonstrates the advantage of using a wide merging chamber to assure that the droplet area is measured far from these edges.

The pixels size of the CCD chip is $7.4 \mu\text{m}$. Since a X4 magnification is used, each pixel measures an area of $1.8 \mu\text{m} \times 1.8 \mu\text{m}$. Therefore each pixel corresponds to a volume of 0.22 picoliter, which is the highest possible measuring resolution of the optical system. In practice, the volume error was two orders of magnitude less precise. It was found that even with a rudimentary cylindrical model, the relative volume error was better than 5%, estimated based on volume conservation (see section below).

b. Analytical expression Vs. semi-experimental approach

Initially an analytical expression for the droplet's volume was derived using Disk Integration, while assuming the droplet has a cylindrical symmetry. In practice we found this method to provide insufficient accuracy. This was due to systematic errors introduced by variations in lighting and image processing filtering parameters which changed between experiments. We attributed an unknown cross-section profile to the droplet edge and derived it together with the edge detection systematic error. This was done by approximating the volume deviation from the cylindrical model by multiplying this residual cross section by the droplet perimeter. Mathematically, we assumed $V = Ah + \alpha\sqrt{A}$, where the parameter α was found experimentally by imposing volume conservation before and after coalescence:

$$hA_1 + \alpha\sqrt{A_1} + hA_2 + \alpha\sqrt{A_2} = hA_f + \alpha\sqrt{A_f}$$

Here A_1 , A_2 are the droplet areas before coalescence and A_f is the droplet area after merging.

Rearranging, we get:

$$\alpha = \frac{h(A_f - A_2 - A_1)}{\sqrt{A_1} + \sqrt{A_2} - \sqrt{A_f}}$$

For different droplet volumes using the same set of measurements, we found α with a small deviation which confirmed this semi-experimental model.

c. Error estimation

After reducing the systematic errors as described above, the error estimation main contribution was due to the term Ah . The ratio of the relative errors of h and A was found to be $(\delta h/h)/(\delta A/A) > 3$, therefore allowing the $\delta A/A$ term to be neglected.

The mixing ratio is given by $r = V_1/V_2$ and is therefore a factor of $\sqrt{2}$ larger than that the relative error in the volume. Therefore $\delta V/V \sim 1.4\%$ and $\delta r/r \sim 2\%$.

References

- 1.J. Shemesh, A. Bransky, M. Khoury and S. Levenberg, *Biomed Microdevices*, 2010, **12**, 907-914.
- 2.A. Bransky, N. Korin, M. Khoury and S. Levenberg, *Lab Chip*, 2009, **9**, 516-520.
- 3.J. Xu and D. Attinger, *Lab Chip: Chips & Tips*, 2008, **22 October 2008**.