

## Supplementary multimedia files

### S1: Mixing inside femtolitre droplets produced by step-emulsification

Caption: femtolitre droplets composed of fluorescein (pH4) and a basic buffer are produced by step-emulsification. The increase in fluorescence results from fluorescein deprotonation when mixing with a basic buffer. The resulting mixing time is 45  $\mu\text{s}$ . Slowed down 1200x.

Keywords: mixing, femtolitre, droplets, step emulsification

### S2: Mixing inside picolitre droplets produced by step-emulsification

Caption: picolitre droplets (fluorescein pH4 + basic buffer) are produced by step-emulsification. Picolitre droplets can be tracked individually and we record their increase in fluorescence. The resulting mixing time is 330  $\mu\text{s}$ . Slowed down 600x.

Keywords: mixing, picolitre, droplets, step emulsification

### S3: Dielectrophoretic sorting of femtolitre droplets

Caption: without electric field, droplets are driven towards the wide channel. In presence of a 700V voltage, DEP forces attract droplets towards the narrow, hydrodynamically unfavorable, channel. Slowed down 5x.

Remark: when laterally confined (0V and 700V), optical effects make droplets look doubled because of the use of high magnification objectives; part of the droplets is out of focus, resulting in different contrasts.

Keywords: dielectrophoretic, sorting, droplets, femtolitre

### S4: Electrocoalescence of femtolitre droplets

Caption: subjected to an electric field, 25 fL droplet pairs reorient along the field direction and coalesce. Two cases are presented: coalescence before the restriction (Position B), predominant at high fields, and coalescence at the restriction (Position A), predominant at low fields. Voltage is here 600V. Slowed down 30x.

Keywords: electrocoalescence, droplets, femtolitre

### S5: Splitting of femtolitre droplets

Caption: femtolitre droplets are sent against a T junction. In the case of small droplets, they direct towards one arm of the junction, without splitting. Above a threshold ratio  $l/w = 3$ , droplets split into two daughter droplets. Slowed down 600x.

Keywords: splitting, droplets, femtolitre

### **S6: Parallelized production of femtolitre droplets**

Caption: 65 fL droplets are produced in a parallelized geometry. The aqueous phase flows through an array of 37 thin channels (1  $\mu\text{m}$  height \* 5  $\mu\text{m}$  width), and falls into a deep oil reservoir (35  $\mu\text{m}$  height). Droplets are produced at a total frequency of ~10 kHz.

Keywords: parallelized production, droplets, femtolitre, step emulsification

### **S7: Reinjection of femtolitre droplets**

Caption: reinjection of 65 femtolitre droplets that were stored off-chip in an Eppendorf under a heavy mineral oil cap for 24 hours. Channel height is 1  $\mu\text{m}$ , enabling the stable reinjection of droplets in a 5  $\mu\text{m}$  wide channel, accessible by standard lithography protocols. Slowed down 300x.

Keywords: reinjection, droplets, femtolitre, off-chip incubation

### **S8: Fabrication of the molds by multi-layer soft lithography**

Caption: we describe a very fast and precise method to create multiple-layer wafers. Tape is used to protect the alignment structures, enabling the fabrication of high thickness ratios between the resist layers.

Keywords: soft lithography, multiple layer, tape