Supporting information of 'A passive microfluidic system based on step emulsification allows to generate libraries of nanoliter-sized droplets from microliter droplets of varying and known concentration of sample'

W. Postek, T.S. Kaminski and P. Garstecki

Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland.

Dimensions of geometry of *DropChop* without bypassing channels

Cross section of the main channel 400x400 μm; tested incline of the slope: a) 10 b) 2.5 degrees; height of the slit directly upstream of the step 70 μm; cross section of the channel downstream of the step 400x400 μm.

Dimensions of geometry of *DropChop* with bypassing channels

Cross section of the main channel 400x400 μm; incline of the slope: 2.5 degrees; height of the slit directly upstream of the step 70 μm; cross section of the channel downstream of the step 400x400 μm; cross section of a bypassing channel: 220 (width) x 200 (depth) μm; cross section of the entrance to a bypassing channel: 220 (width) x 50 (depth) μm.

Dimensions of geometry of the integrated device

Cross section of the main channel upstream of the slope 400x400 μm; incline of the slope: 2.5 degrees; height of the slit directly upstream of the step 30 μm; cross section of the channel downstream of the step 200 (height) x 400 (width) μm; cross section of a bypassing channel: 220 (width) x 40 (depth) μm; cross section of the entrance to a bypassing channel: 170 (width) x 50 (depth) μm. Hydrodynamic trap used for diluting rhodamine 110 for analysis of dilution ratio: Length of the trap: 2400 μm; cross section of the main channel: 600 (width) x 400 (depth) μm; depth of bypasses and height of the slit over the barrier in the main channel: 200 μm; length of the barrier: 230 μm.

Polydispersity of emulsions generated at highest tested concentration of fluorosurfactant

The CV of diameters of generated emulsions was ca. 15 % or more (Fig. 3 d) at 1.0 % (w/w) of fluorosurfactant and at the range of tested flow rates from 6 ml/h to 16 ml/h. Frequency of generation of droplets increases as the rear end of the mother droplet is emulsified (Fig. S1) and the average frequency of generation of droplets increases with increasing concentration of surfactant (Fig. 3c). Droplets formed from the rear end of the mother droplet interfere with each other during emulsification (Fig. S2 b) due to high frequency of generation of droplets. At a critical point, the neck does not break anymore (Fig. S2 c) and a large droplet is formed, followed by multiple smaller droplets (Fig. S2 d). At higher flow rates, (i) this large droplet has a bigger volume and (ii) there is higher number of small droplets following the large droplet. Interference of droplets during emulsification is also present at lower concentrations of surfactant at higher rates of flow.

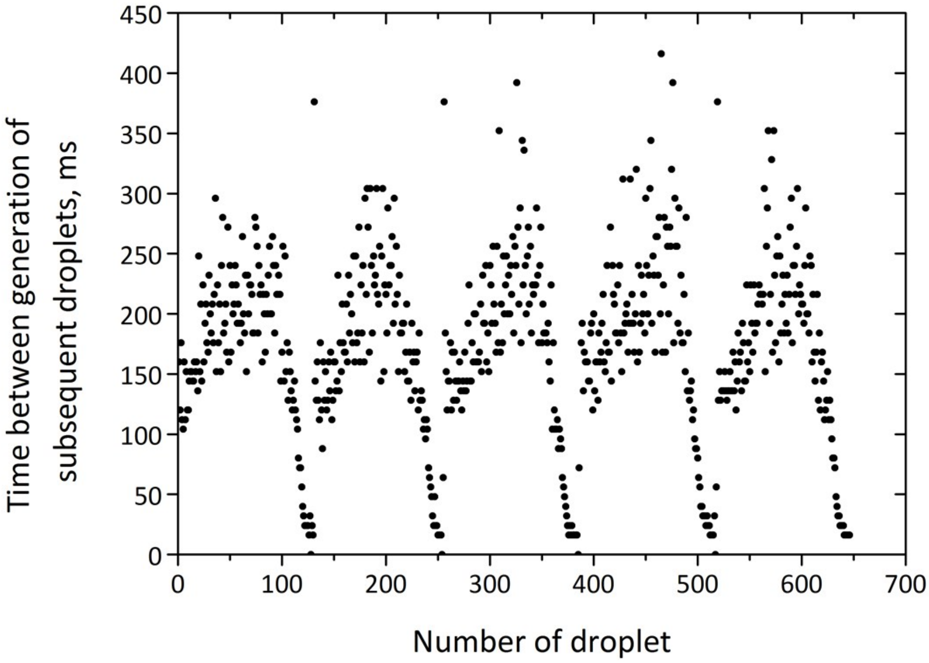

Fig. S1. Time between generation of subsequent droplets for 5 libraries of droplets (periods of time between libraries are omitted).

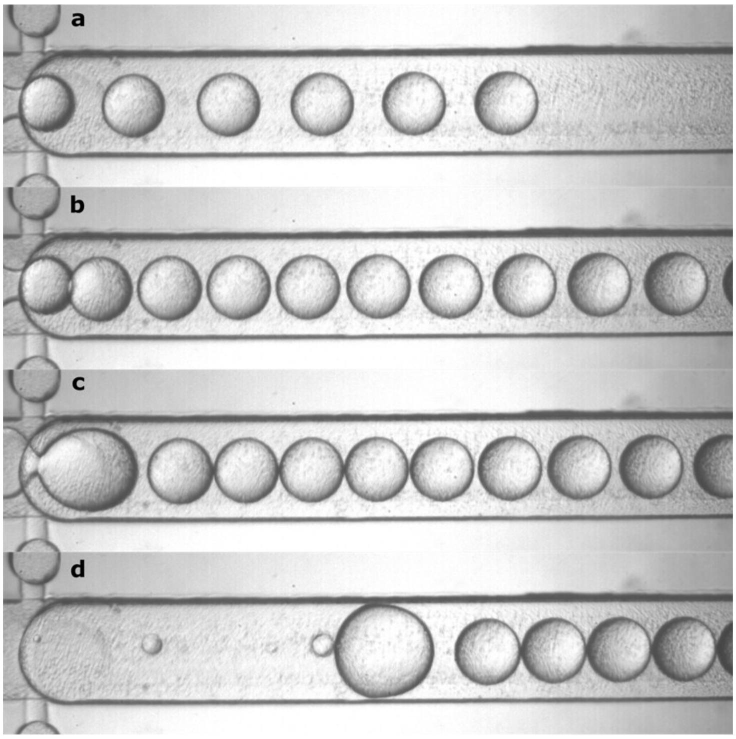

Fig. S2 Formation of the emulsion at 1.0 % (w/w) fluorosurfactant at flow rate of 6 ml/h. a) Droplets generated from the front of the mother droplet do not interfere with each other. b) Droplets generated from the rear end of the mother droplet interfere with each other. c) At a critical point, the neck does not break. d) One large droplet and multiple small droplets are generated in the final stage of the emulsification process.

Generation of larger and smaller droplets in *DropChop* without bypasses

The first droplet of a generated emulsion is pushed by the next generated droplet randomly to the left or the right side of the channel. The droplets formed at the step adjust their position in the channel in respect to the position of the previous droplet: if the first droplet is on the right, the second will be on the left, the third on the right, the fourth on the left etc. Sometimes, mostly at the beginning of the splitting process, the droplets break this pattern, and position themselves e.g. first droplet on the right, then left, then right, then right, then left, then right etc. Droplet in the generated emulsion are densely packed along the main channel – every second droplet occupies the left or the right upper corner of the channel and the other half of droplets occupies diagonally opposite corner. However, droplets occupying the top-most corner, which is more distant from the step, are larger because during formation there is more space available for them just downstream the step (Fig. S3).

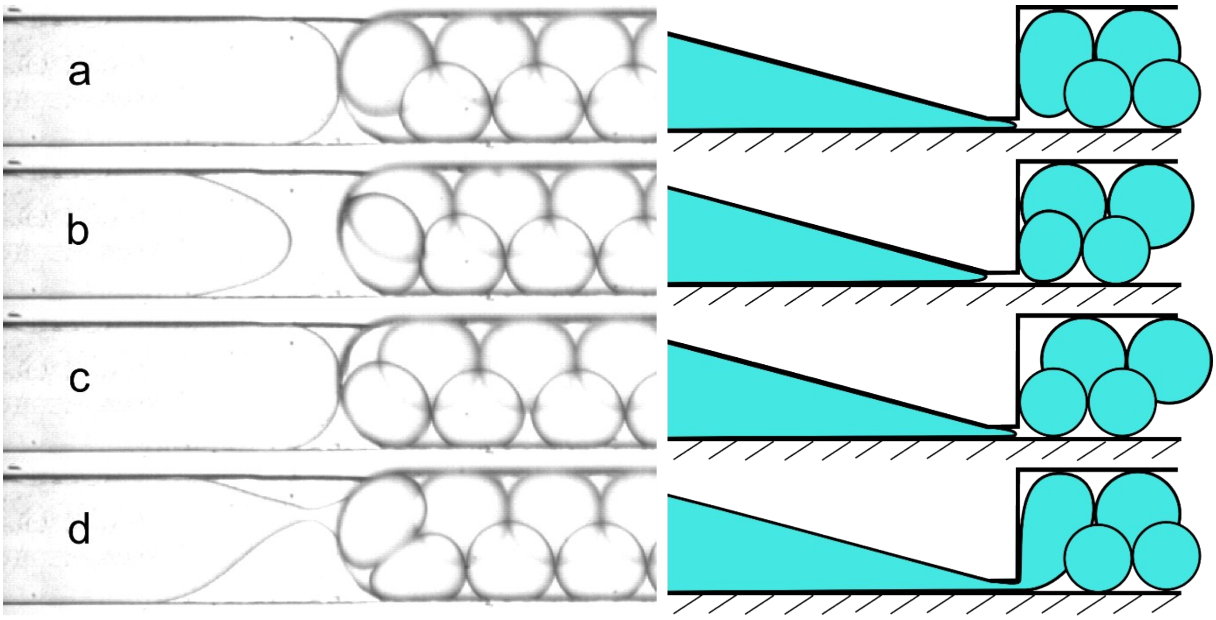

Fig. S3 a) A large droplet is situated above the step. b) A new droplet fills the available space close to the step, becoming a small droplet. c) A small droplet leaves more space for the new droplet. d) A new droplet fills the available space above the step, and becomes a large droplet. Cartoons on the right portrait the side view of the process at the step.

Supplementary movie S1

The recoding depicts breakup of a droplet from a mother droplet at *DropChop* with bypasses. Formation of neck is visible before breakup, suggesting that the regime of breakup is step emulsification rather than flow focusing. The video had been recorded at 10k frames per second, and was later sped up 3 times for clarity.

Supplementary movie S2

The recording depicts emulsification of a mother droplet at *DropChop* with bypasses. Changes in frequency of generation of droplets visible. Last droplet of the generated emulsion is visibly smaller than the rest of the droplets in the generated emulsion. The recording is presented in real time.

Supplementary movie S3

The recording depicts generation of mother droplets of diluent at T-junction, dilution at the trap-based dilutor, mixing of content of droplets and emulsification at *DropChop* with bypasses. Dilution ratios calculated and described in the article text were based on measurements done on droplets diluted at a hydrodynamic trap presented here. The recording is sped up 6 times in relation to real time.

Supplementary movie S4

The recording depicts emulsification of droplets at *DropChop* in the integrated device. Between each mother droplet, 30 seconds of recording was cut out, as in this time the mother droplets moved from the T-junction to *DropChop* and were out of the area that was recorded. For this recording, we used diluting trap of dimensions different than the trap we used for final measurements of dilution ratio we described in article text. The rest of the dimensions in the device depicted here is as described in ‘Dimensions of geometry of the integrated device’. The recording is presented in real time.