Supplementary Information for

Microfluidic screening of antibiotic susceptibility at a single-cell level shows inoculum effect of cefotaxime in *E. coli*

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> Videos S1 to S3
> CAD designs of the generation chip and the detection chip in .dxf format

Supplementary Information Text

Geometry of the nozzle. Initially, we wanted to generate emulsions with previously published geometries for emulsification of large droplets, such as a flow-focusing module[1,2] or a *DropChop* geometry with bypasses[3], but the emulsions produced in the aforementioned geometries proved to be inadequate for our goals, since emulsions generated with a flow-focusing geometry or with a *DropChop* geometry with bypasses had a low water to oil volume fraction. To increase the packing density inside tankers, we used *DropChop* without bypasses, and to enhance the removal of freshly generated droplets from the area immediately downstream of the step, we set the device with *DropChop* vertically, so that the escape of droplets from the vicinity of the step is assisted by buoyancy, similarly to what was shown elsewhere.[4–6] Removing of droplets from the step is crucial to generation of a monodisperse emulsion with step emulsification, and this matter was covered more extensively before.[3,4] Emulsion formed at a vertically oriented *DropChop* were highly polydisperse (ca. 27 % coefficient of variance of volumes) at frequency of generation of droplets of 75 Hz (Fig. 2a). This is in concordance with what we showed before[3]: the higher the concentration of surfactant in the continuous phase, the higher the polydispersity of emulsions generated at *DropChop*. Note that we have used 2.0 % (w/w) of EA surfactant here, and in our previous report we have not used more than 1.0 % (w/w).

We introduced a pillar in the constriction directly upstream of the step (Fig. S1a) to form two nozzles, and consequently reduce the speed of incoming mother-droplet per nozzle. We chose the geometry of the pillar to be a triangle, as this was before suggested to be an optimal geometry of a nozzle for step emulsification[7]. We observed that introduction of a pillar reduced the volume of the generated droplets (Fig. S1b): from an average 4.81 ± 0.76 nl to 2.36 ± 0.28 nl (for nozzle constriction height of 40 µm), also we noted a reduction of the coefficient of variance of volumes of the generated droplets from 16 % to 12 %. The smaller droplet sizes are most probably linked to the increased curvature of the droplets at a nozzle with the pillar. In this geometry the height of the constriction upstream of the step was 40 µm. For the rest of the experiments (also in the main article), we used the geometry with a triangular pillar with the height of the constriction upstream of the step of 30 µm to reduce the volume of the generated droplets.

Stability of tankers. To test whether the droplets inside tankers coalesce over time, we performed qualitative and quantitative experiments. For the qualitative test, we generated emulsions of droplets of MH and bacteria at three different fluorosurfactant concentrations (0.5 %, 1.0 %, and 2.0 % <w/w>). We used yellow fluorescent protein (YFP) producing *Escherichia coli* strain in this and all the following experiments. After incubation at room temperature for 16 hours, we visually inspected the tankers. It was visible with naked eye that coalescence occurred in droplets generated at 0.5 % (w/w) of fluorosurfactant (Fig. S2a). It was, however, not obvious if the droplets coalesced at higher tested concentration of fluorosurfactant (Fig. S2b-c). To test if we could detect coalescence in emulsions, we generated tankers that contained large droplets (average size of droplets was 1.8 nl) at 1.0 % (w/w) of fluorosurfactant, and we measured the sizes of droplets from 5 tankers at the detection chip. We then stored the remainder of the generated tankers overnight at room temperature for 16 hours. Then, we measured 5 of the remaining tankers. We could detect a new population of droplets that was twice the volume of the majority of droplets in the emulsions (Fig. S2d-e). We assume that this new population of droplets is formed by droplets that coalesced overnight. We then proceeded to generation of tankers that contained droplets of MH medium with bacteria. We then incubated the tankers at 37ºC for 16 hours. After incubation, there was no new population of large droplets (Fig. S2f-g), therefore we assume that there was no coalescence of droplets during incubation.
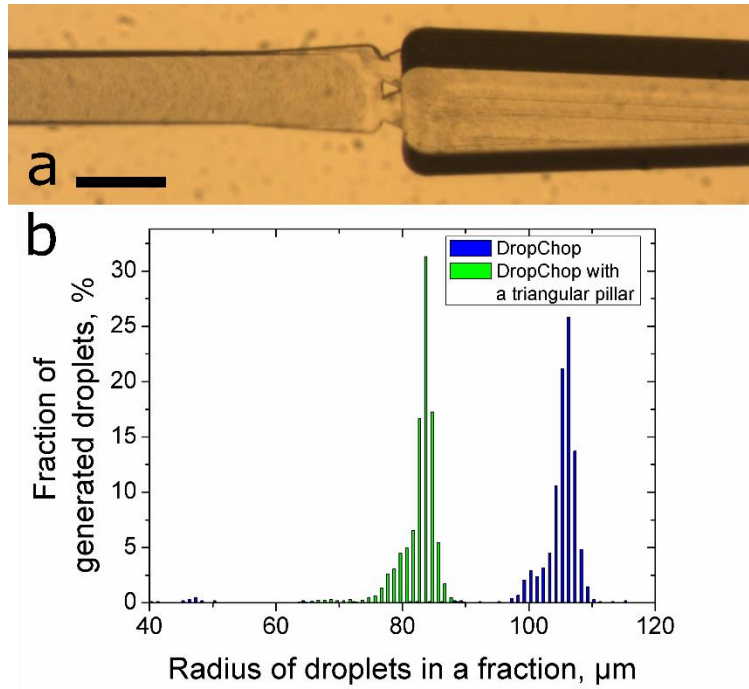
Fig. S1 Modified *DropChop* geometry and the generated emulsions. a) isometric photography of the modified *DropChop*. The flow is realized from left to right. A sloped constriction leads mother-droplets from the left to the constriction with a triangular pillar. The droplets are formed from the two nozzles and removed from the constriction by buoyancy. Height of the constriction is 30 µm, the slope is of an angle of 2.5 degrees, the chamber in which the generated emulsion is stored is 400 µm deep. The width of the nozzle at the transition between the constriction and the collection chamber is 200 µm. The entrances to the nozzles are 100 µm wide each, the base of the triangle is 100 µm wide, the height of the triangle is 140 µm. b) Histograms of sizes of droplets generated at different *DropChop* geometries with nozzle constriction height of 40 µm. Addition of a pillar results in smaller generated droplets.
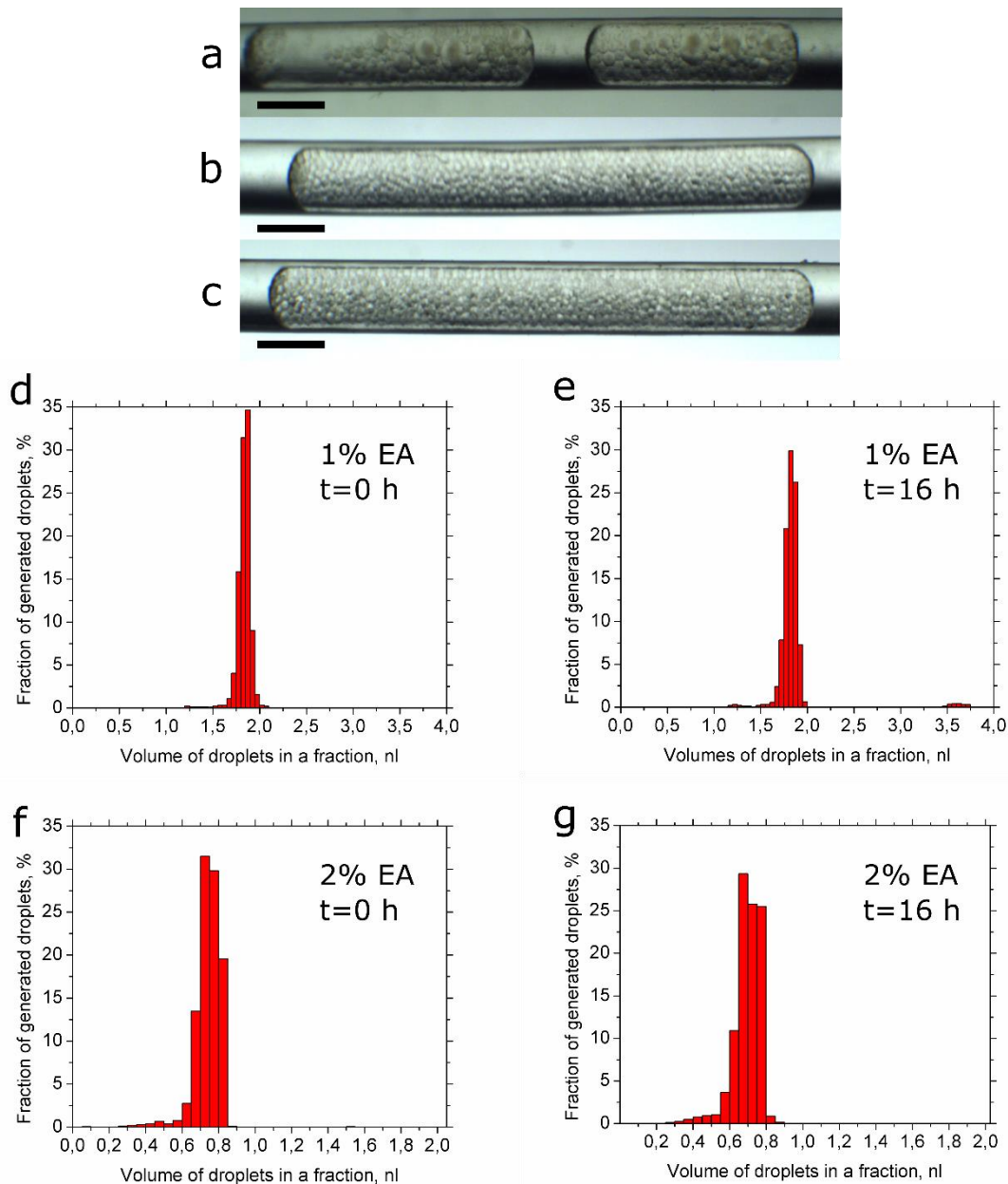
Fig. S2 Stability of tankers. a-c) qualitative assessment of stability after 16 hours at room temperature at different content of EA surfactant in the continuous phase: a) 0.5 % w/w; b) 1.0 % w/w; c) 2.0 % w/w. It is not possible to assess the stability of emulsions in cases b and c. d-g) quantitative analysis of stability of tankers. d-e) water droplets generated to test if the assessment of coalescence of droplets is possible: large water droplets were generated at 1.0 % w/w of surfactant and left for 16 h at room temperature. After 16 h a new population of large droplets is visible, indicating coalescence. f-g) Stability of tankers of smaller droplets at 2.0 % w/w of surfactant in the continuous phase. No new populations of droplets emerged after incubation of the tankers for 16 h at 37°C.
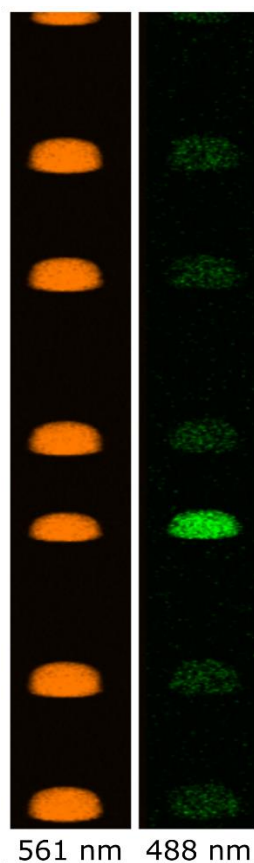
561 nm   488 nm

Fig. S3 Detection of fluorescence from single droplets during the contamination experiment. Left: the red fluorescence (rhodamine) channel. Right: the green fluorescence (fluorescein) channel. For detection of signals, the tankers are placed in a detection chip, which contains a broad and deep channel that narrows down gradually, so that at one point only a single droplet can fit this channel. Then, additional fluorinated oil phase is used to space the droplets from each other. The droplets are then scanned in one line, hence the picture shows kymographs of droplets flowing from bottom to top of the picture. In this contamination experiment, the red dye marked every droplet, and the green dye marked contaminating droplets.

Video S1. Generation of emulsion at a modified *DropChop* geometry and separation of emulsions with squalane. (speed 2.5x)

Video S2: Transfer of an emulsion from the generation chip to the tubing. (real time)

Video S3: Transfer of a tanker from the tubing to the detection chip. (speed 2x)

References

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