Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary information

Influence of electric-field assisted sorting on viability of Streptomyces puniceus

After being suspendend in MMM-soft agar, exposed and non-exposed (control) droplets were spread on MMM-agar plates (s. Material and Methods). Colonies were counted after three days incubation at 28 °C (Table S1). Apart from a slight growth delay of ~ ½ day, colonies from exposed droplets developed normally (Fig. S1).

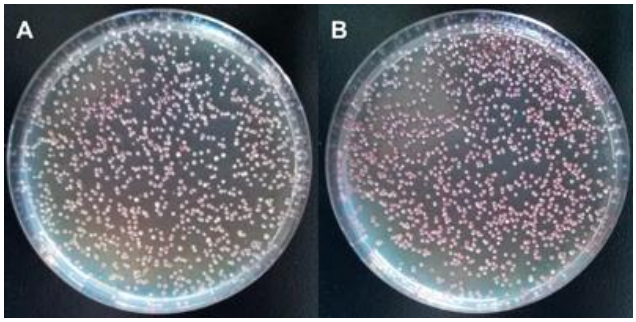

Fig S1: MMM-agar dishes with colonies originating from exposed (A) and non-exposed (B) droplets

The soft-agar technique was applied to ensure gentle application of the microcolonies to the plates. Any disruption of the droplet-confined colonies might lead to undesired deviations by multiplication of colony forming units (CFUs) found on the agar plate. Since this technique is yet not validated and replicates are missing, the results presented here are only of qualitative value. It is demonstrated, that the randomly chosen *Actinobacteria* strain survives the process of electric field-assisted sorting. This justifies the assumption, that on-chip sorting of droplet-confined *Actinobacteria* is generally feasible. However, the CFUs on the control plate are slightly higher (1518) than on the plate with non-exposed droplets (1312). Together with the observed delay in growth, this might indicate detrimental effects of sorting on the tested strain. Nevertheless, future experiments with a larger variety of tested strains will allow for a more detailed view on the influence of electric field-assisted sorting on *Actinobacteria*. If significant detrimental effects are observed, sorting the empty droplets instead of the colonized ones might facilitate maintaining high enrichment rates in future experiments.

Image stacks of DoG-filter evaluation

A set of 500 triggered images was chosen for the assessment of sensitivity and specificity of mycelia detection for three different parameter settings of the DoG filter (s. Results and Discussion). The outcome of each single droplet evaluation by the DoG filter is indicated by a magenta ("positive") or cyan ("negative") droplet boundary. Manual validation was accomplished through independent investigation by four experts.

Supplementary movies

Movie S1: Video of germination-dependent droplet sorting

The supplementary video shows a sequence recorded during growth-dependent IDS at 5-fold magnification with ~100 Hz. The according triggered images of each passing droplet are displayed within the white frame over the actual region of imaging. Droplets identified as colonised ("positive") are marked with magenta boundaries, empty droplets ("negative") with cyan boundaries. In a few cases of droplets categorised as negative, the triggered images show very faint mycelial structures, implying false negative droplet classification. Yet, most false negatives are caused by hyphae located in a different focal plane upon triggered imaging. A very clear case of such a false negative can be observed for the droplet, which is displayed in the trigger image frame at 54 s. Although the droplet is recognised as empty, confined mycelial structures are clearly observed further downstream. This issue can be easily tackled using a decreased channel depth.

Movie S2: Video of picoinjection to colonised droplets

Droplets were incubated and reinjected as described. The flow of the dispensing channel was adjusted to 25 nL/s. An AC-potential of 30 V (peak to peak) was applied across the electrodes.