DETERMINISTIC LATERAL DISPLACEMENT DEVICE FOR DROPLET SEPARATION BY SIZE – TOWARDS RAPID CLONAL SELECTION BASED ON DROPLET SHRINKING

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ABSTRACT
We present a novel method for robust passive separation of microfluidic droplets by size using deterministic lateral displacement (DLD). We also show that droplets containing Saccharomyces Cervisiae shrink significantly during incubation while droplets containing only yeast media retain their size. We demonstrate the DLD device by sorting out shrunken yeast-cell containing droplets from a 40-fold excess of ~33% larger yeast-cell-free droplets generated at the same time, suggesting that DLD might be used for clonal selection. The same device also separates 11μm from 30μm droplets at a rate of 12000 droplets/second, more than twofold faster than previously demonstrated passive hydrodynamic separation devices [1].

KEYWORDS: Droplets, Separation, High-Throughput, Cells

INTRODUCTION
Droplet microfluidics is increasingly being used in complex manipulations, which can yield polydisperse emulsions. Subsequently regaining monodisperse emulsions after droplet fusion, heating induced coalescence in e.g. PCR or other droplet manipulations resulting in polydisperse emulsions can be useful or necessary for further downstream processing. In a recent publication this issue has been addressed through hydrodynamic passive separation in a trident channel at rates <4500Hz [1]. Here we apply the DLD concept to this problem, increasing throughput more than twofold and at the same time demonstrating the possibility of using DLD to separate highly deformable particles, i.e. microfluidically generated droplets. DLD is a high throughput size separation method which has been shown to separate solid micrometer sized particles with a tolerance of less than 10 nm [2] utilizing a tilted pillar array. In DLD devices, objects are separated depending on a critical diameter (DC) which depends on the design parameters of the array, shown in Figure 1 and described by the equation DC=2Gd/w, where η is the parabolic flow correction factor.

S.Cervisiae is a common biotechnological model system and is used in bioproduction of e.g. fuel ethanol [3] and for production of specific biomolecules, such as the antimalarial drug precursor artemisinic acid [4], using strains specifically engineered by synthetic biology. One approach to selecting novel yeast strains with specific biological properties is through directed evolution, recently demonstrated in microfluidic droplets using a fluorogenic substrate and serial dielectrophoretic sorting to select the strains producing the most efficient enzyme from a genetic library at high throughput [5].

EXPERIMENTAL
Microfluidic devices were manufactured in Polydimethylsiloxane by standard soft lithography. Aqueous droplets were generated by pressure driven flow in a fluorinated carrier oil (HFE 7500, 3M) stabilized by a surfactant (EA, Rain-Dance Technologies) in the oil phase. The DLD circuit can generate 11 and 30μm aqueous droplets or allow input of pre-generated emulsions. Droplets were injected at the center of a large channel with a tilted pillar array with 60μm cylindrical pillars separated by gaps of 60μm and a period of 10 rows (Figure 2). Oil was injected from the two side inlets O1-2 at flow rates of 5ml/hr generating a laminar flow along the main channel. At the outlet reservoir (EC) a row of 9
outlets arranged perpendicular to the flow direction connected to emulsion collection vials. The resulting device has a theoretical critical diameter for separation of 24μm (for η=2). The depth of all channels was 30μm. 50μm droplets containing on average two and 30μm droplets containing on average 0.03 S. Cervisiae cells in YPD medium were generated in a separate device and incubated for 8 or 36 hours respectively at 30°C. After incubation most of the 30μm yeast/YPD emulsion was injected into the DLD device for separation while a small subset was re-injected into an analysis circuit [6]. Here droplets were injected with additional oil and individually passed through a laser. Fluorescence, i.e. the autofluorescence of the yeast media, was sampled using a photomultiplier tube to determine relative droplet size.

**RESULTS AND DISCUSSION**

Operating the DLD chip at moderate flow rates yielded 12000 and 200 droplets per second of the 11μm and 30μm droplets respectively, thus resulting in a 60-fold excess of smaller droplets. However, this bias in size distribution did not challenge the separation performance; all observed droplets were correctly separated. Upon injection into the pillar array 11μm droplets were observed passing through the array following the direction of the flow while 30μm droplets were seen following the tilt angle of the array (Figure 2) resulting in the complete separation of the droplets into two discrete monodisperse emulsions immediately before entering the outlet reservoir.

The 50μm droplets were imaged before and following incubation (Figure 3). Interestingly, yeast-containing droplets had shrunk substantially while empty droplets remained their original size. Meanwhile the yeast cells had divided.

Figure 4 shows the separation of shrunken droplets by size in a DLD device. The 30μm droplets generated from the yeast containing aqueous phase were monodisperse upon generation but most yeast containing droplets had shrunk to an average 22.5μm following incubation. The ratio of shrunken droplets to those retaining their original size was measured by laser induced fluorescence to be 2.5/100. From this starting material shrunken droplets were concentrated and collected at a high purity (Figure 4G).
CONCLUSION

We demonstrate high throughput separation of microfluidically generated droplets in a DLD device at a rate of 12000 droplets per second. We also observe the selective shrinking of yeast containing droplets and separate these droplets by size based on this biological determining factor, separating a droplet population with a mean diameter of 22.5 μm from a population of 33% larger diameter present at a 40-fold excess. We are now characterizing the device more precisely using higher separation rates as well as investigating the efficient selection of yeast strains based on biological characteristics by separating shrunken droplets.

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