RAPID AND CONTINUOUS MAGNETIC SEPARATION IN DROPLET MICROFLUIDIC DEVICES

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ABSTRACT

This paper presents a droplet microfluidic method to extract molecules of interest from a droplet in a rapid and continuous fashion. We accomplish this by first marginalizing functionalized superparamagnetic beads in within the droplet using a magnetic field, and then splitting the droplet into one droplet containing the majority of magnetic beads and one droplet containing the minority fraction.

A similar enrichment strategy have been reported in the recent literature [1, 2] using a 50:50 t-shaped splitting junction but a quantitative analysis and optimization of the factors that affect the enrichment efficiency was lacking. In contrast, we quantitatively analyzed the factors that affect the efficiency of marginalization and droplet splitting. We also improved the design of the splitting junction to allow the preferential extraction of bead-rich regions of the droplets resulting in a 5x enrichment of magnetic beads during a single pass.

KEYWORDS: Droplet Microfluidics, magnetic, separation, single-cell genomics

INTRODUCTION

In the past decade, droplet microfluidic technology has experienced tremendous growth [3, 4] and has been used to develop a wide range of applications such as: enzyme evolution, drug screening, genetic analysis, and single-cell and organism analysis. These applications have been enabled mostly by the development of robust methods at high-throughput that allow controlled droplet generation, fusion, injection, on-chip incubation, sorting, and splitting.

Despite its benefits, the droplet format inherently limits the ability to effectively concentrate or extract target molecules. These operations are for instance critical to developing high-throughput methods to extract mRNA from single cell lysates for analysing the gene expression of single cells. Magnetic bead extraction from within droplets is achieved by marginalizing beads inside droplets with a magnetic field and splitting the droplets to concentrate the beads within a daughter droplet [1, 2]. In this paper we perform a quantitative analysis and optimization of the factors that affect the enrichment efficiency, and propose an original method to perform droplet splitting that enables the preferential extraction of bead-rich regions. Our motivation is to study the effect of experimental parameters such as magnet strength and position, droplet velocity, and the design of the splitting fork on the enrichment of magnetic beads within microfluidic droplets. As we will illustrate, this system exhibits a complex coupling between internal flow fields and the forces acting on the magnetic particles.

EXPERIMENTAL

For our experiments we fabricated hybrid PDMS/Glass chips using soft-lithography. The microfluidic setup is based on an inverted microscope (Motic, AE31) with camera and LED illumination that allows stroboscopic illumination to measure droplet velocities. We used 1μm superparamagnetic beads (Oligo d(T)25 Magnetic beads, New England Biolabs). Droplets are stabilized in HFE7500 fluorinated oil using a PEG-Krytox surfactant at 1% concentration.

RESULTS AND DISCUSSION

We first identified the optimal position of the splitting fork. We flowed droplets that contain magnetic beads past a permanent magnet at low velocity to approximate quasi-static conditions and neglect flow recirculation within droplets. Fig.1 shows micrographs of droplets in these conditions as well as magnetic field lines and the magnitude of the magnetic flux as function of position relative to the

magnet. We found that marginalization is optimal at the midline of the magnet and that marginalization is a good predictor of bead enrichment through splitting at low to moderate droplet velocities.

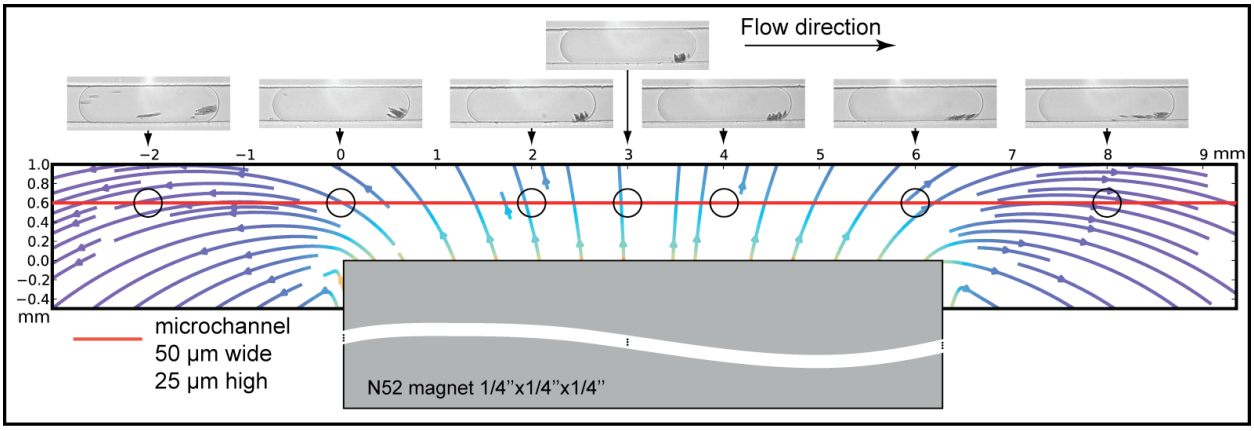

Figure 1: Magnetic particle distribution and orientation as a function of position relative to the magnet. Particle aggregates are most compact when droplets reach the midsection of the magnet. Bead distribution is not mirror symmetric with respect to the magnet midsection because of internal flow fields. Microscopic pictures were taken at low velocity (0.8 mm/s) to approximate quasi-static conditions, with the N52 ¼" cubic magnet located at 635µm from the channel.

We further characterized the effect of the coupling of magnetic and hydrodynamic forces on particle distribution by quantifying the partitioning of magnetic particles within droplets. In Fig.2, we report the fraction of beads located in the mid-section away from the magnet (top region) as a function of droplet velocity and magnetic strength that is modulated by varying the distance of the magnet to the channel. Above a critical droplet velocity, which depends on the magnetic force, some beads start accumulating into the top back corner while the bulk of beads remain in the lower front corner. Clearly, the higher the magnetic field strength the faster we can flow droplets to achieve the same bead distribution.

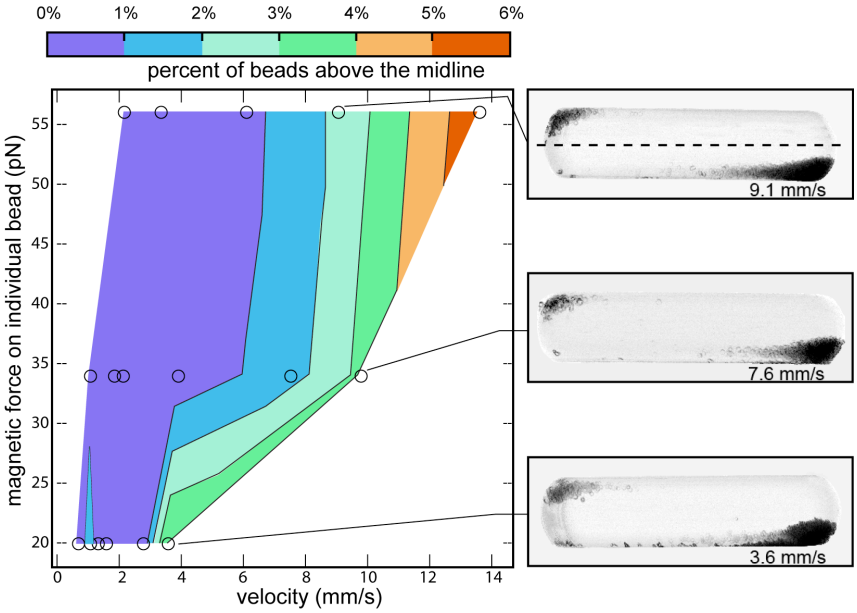

Figure 2. Contour plot of the partitioning of magnetic particles plotted as percentage of magnetic beads above the midline (see top insert) as a function of droplet velocity and magnetic force. The circles represent the experimental points. The contour plot was created by linear interpolation in force and velocity. Inserts illustrate similar bead partitioningfor different magnetic forces. Each mage is the minimal projection of at least 45 individual droplets.

Finally, by manipulating channel resistance and geometry, we designed asymmetric splitting forks that direct (or control) the preferential extraction of bead-rich regions of the droplets. We accomplished this by manipulating the ratio of the hydrodynamic resistances of the two fork branches as well as the geometry of the splitting fork. Fig. 3 shows the splitting fork designs and the measured splitting profiles. Using this design we were able to improve the enrichment of magnetic beads from 1:1 to 1:5 at similar bead loss percentages.

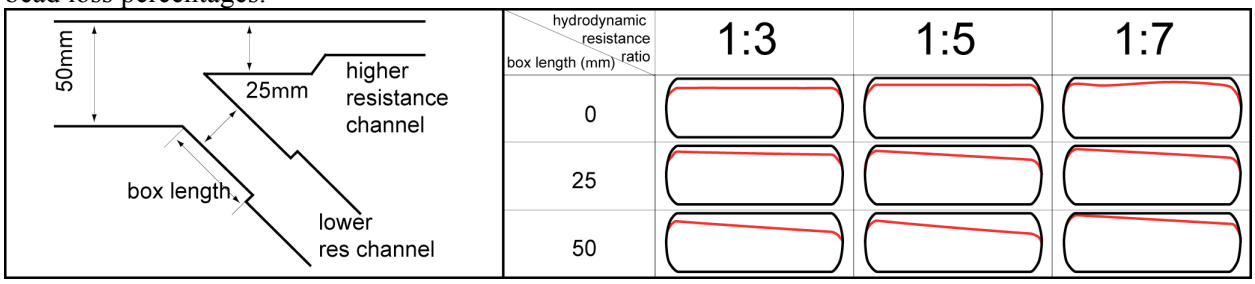

Figure 3: Tailoring the splitting profile. a) The design of the splitting fork consists of two branches of different hydrodynamic resistances where we incorporated an initial symmetrical box to favor the separation of the lower front region of droplets. b) Splitting profiles of different designs show that the symmetrical box has an effect on droplet splitting and permits to comparatively collect more of the region of interest versus the back of droplets which is devoid of magnetic beads.

CONCLUSION

We anticipate that our separation technology is well suited for applications in single-cell genomics and proteomics. In particular, our method could be used to separate mRNA bound to poly-dT functionalized magnetic microparticles from single cell lysates to prepare single-cell cDNA libraries.

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