HIGH-THROUGHPUT PATTERNING OF SINGLE MAGNETIC BEADS USING DIGITAL MICROFLUIDIC TECHNOLOGY

Daan Witters[1], Karel Knez[1], Kris Janssen[1], Bert Verbruggen[1], Robert Puers[2], and Jeroen Lammertyn[1]
[1]*BIOSYST-MeBioS, KU Leuven, Belgium*
[2]*MICAS-ESAT, KU Leuven, Belgium*

ABSTRACT

We present a novel strategy for patterning single magnetic beads by using digital microfluidics (DMF). A droplet containing a suspension of superparamagnetic particles is transported back and forth over an array of femtoliter-sized microwells by using electrowetting-on-dielectric actuation forces. Compared to existing methods, this technique allows patterning of superparamagnetic beads in microwells with unprecedented high loading efficiencies (>95%) and a high single-bead per microwell resolution, while combining high-throughput printing of particles with a high degree of flexibility. This novel approach for creating highly ordered particle arrays can greatly enhance their application in particle-based bio-assays.

KEYWORDS

Digital Microfluidics, Electrowetting-on-Dielectric, Magnetic Beads, Bead Patterning, Micro- and Nanoparticle Patterning

INTRODUCTION

Well-ordered patterns of micro- to nanometer-sized particles have become an important tool for performing a wide range of applications including protein immunoassays, gene expression and genotyping [1]. Patterns of single superparamagnetic beads have for instance been applied to perform digital enzyme-linked immunosorbent assays (ELISAs), where single beads containing biomarkers are ordered and isolated in single femtoliter-sized wells [2]. Bead-based bio-assays rely heavily on the ability of miniaturized systems to capture, pattern, and expose micro- and nanometer sized beads for a wide variety of applications. Moreover, in cutting-edge materials science, particle patterns are playing a crucial role for nano- and optoelectronic device fabrication [3].

Common existing microfluidic approaches for patterning particles usually rely on centrifugal, dielectrophoretic or hydrodynamic trapping of beads [1]. Here, we present DMF as a promising platform for patterning superparamagnetic microparticles. DMF enables the manipulation of discrete droplets on arrays of actuation electrodes covered with a hydrophobic dielectric layer by using electrostatic actuation forces. Recently, we demonstrated how DMF could be used for high-throughput printing of large arrays of femtoliter droplets by transporting larger mother droplets over hydrophilic-in-hydrophobic micropatterns [4]. The selective wettability of the hydrophilic micropatches compared to the hydrophobic Teflon-AF background matrix causes femtoliter-sized droplets to be deposited inside of these micropatches. Here, we use this concept to assemble dense, high-resolution patterns of single magnetic particles by confining them to femtoliter volume chambers.

EXPERIMENTAL

DMF chips were fabricated in-house as described previously [5], and consist of a double-plate chip design (Figure 1). For creating microwells suitable for single particle patterning, the top plate of the device consisted of an ITO-coated glass slide (Delta technologies) that was coated with 3.25 μm of Teflon-AF (Dupont). Subsequently, this Teflon-AF layer was covered with a protective Parylene-C mask (500 nm thickness) via chemical vapor deposition (AL 200, Plasma Parylene Coating Services). This latter Parylene-C mask protected the hydrophobicity of the Teflon-AF layer which is crucial for DMF as it enables efficient droplet manipulations. An aluminum hard mask was deposited on top of this assembly and was patterned by photolithography using AZ-1505 positive photoresist (AZ Electronic Materials) and aluminum wet etching using Transene type A aluminum etchant. Using reactive ion etching with O_2-plasma, the exposed underlying polymer layers (Parylene-C and Teflon-AF) were etched away. After etching away the remaining aluminum hard mask and mechanically peeling off the Parylene-C mask, 65000 microwells of 4 μm diameter and 3.25 μm depth in Teflon-AF were obtained. The top plate containing this array was then placed on top of the bottom plate containing buried electrodes, while spacers (80 μm thickness) separated both plates. Droplets were sandwiched between both plates and were actuated by means of electrowetting-on-dielectric (EWOD) with high-voltage driving potentials of 140 V_{DC}. Finally, a permanent magnet was placed on top of the array containing femtoliter-sized microwells (Figure 1) in order to speed up bead transfer and bead trapping inside the microwells.

Streptavidin-coated superparamagnetic beads of 2.8 μm diameter (Dynabeads M-280 Streptavidin, Life technologies) were washed 3 times with PBS buffer and subsequently incubated with biotinylated green fluorescent protein (GFP) for 1 h. Finally, beads were washed again with PBS buffer (3 times) for removing any non-bound GFP. This procedure allowed easy visualization of beads with standard fluorescence microscopy.

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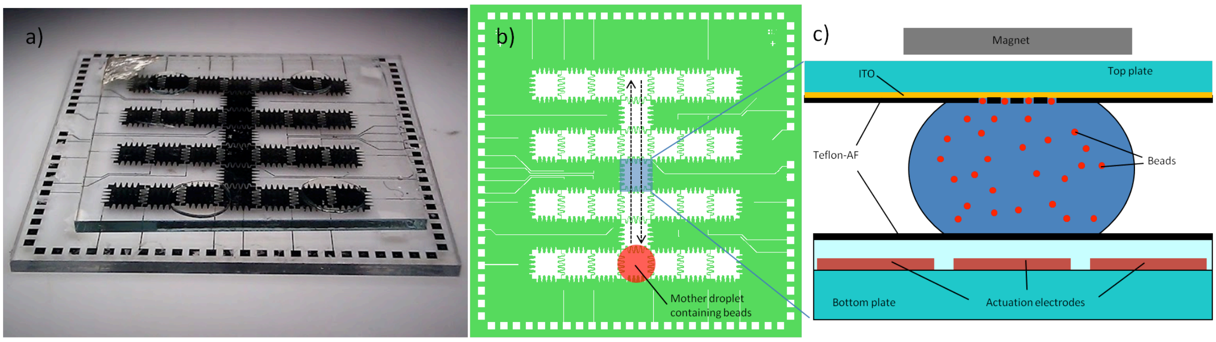

Figure 1. a) Photograph of a double plate digital microfluidic device. b) Scheme depicting the bead-loading principle. A droplet of concentrated bead solution is shuttled back and forth over a capture array of microwells. c) Side view demonstrating bead loading with a magnet positioned on top of the capture array for fast capture of magnetic beads.

RESULTS

Colloidal suspensions of superparamagnetic beads were loaded as 5 µL sized mother droplets on the chip. After placing the top plate containing the microwells on top of the bottom plate, this droplet was transported towards the array. For attraction of beads to microwells, a magnet was placed on the microwell-array (Figure 1). The droplet of concentrated bead-solution was shuttled over the array by using a 140 V actuation voltage. As such, magnetic beads were attracted inside the microwells due to the magnetic force, thereby trapping them inside of the microwells while the drag force of the receeding droplet meniscus removes excess beads off the Teflon-AF surface. Although the mother droplet was slowed down by the presence of the array for bead-trapping, droplet movement was not inhibited when using an electrode activation time of >500 msec. Superparamagnetic beads could effectively be trapped inside the microwells, as demonstrated by scanning electron microscope (SEM) images (Figure 2). Depending on the number of times a droplet was shuttled over the capture array, different bead-loading efficiencies were obtained after 1 min of droplet shuttling (Figure 3). When performing 10 seeding cycles, bead loading efficiencies of >95% were obtained. This loading efficiency is higher than earlier reported results (40-60%) where beads were loaded by using centrifugal forces or allowing beads to settle on the array by means of gravitational forces [2,6]. An improved loading efficiency allows more beads to be interrogated on a smaller surface for optical detection, which is beneficial for a wide variety of bead-based bio-assays. SEM images in Figure 2 also show the high single-bead per microwell resolution.

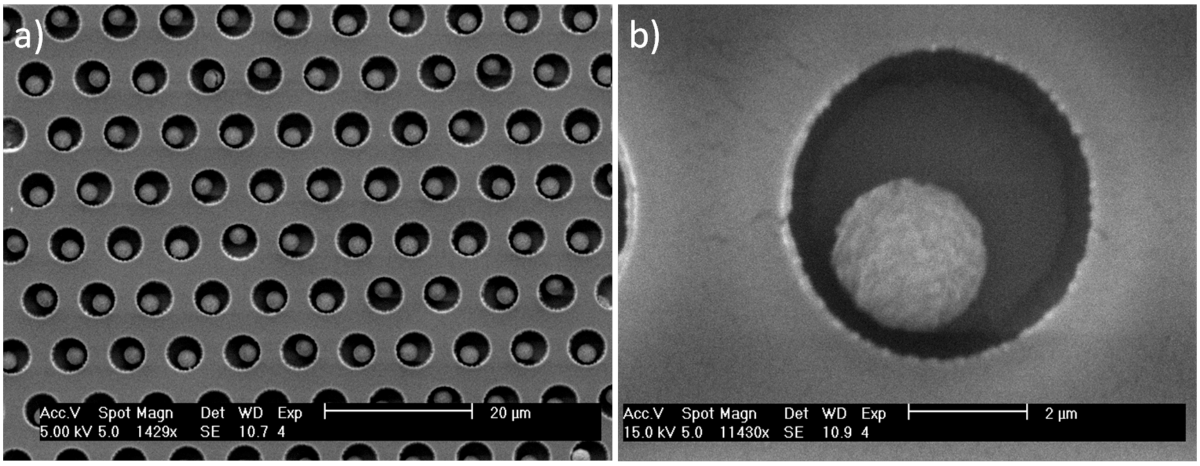

Figure 2. a) SEM image of a part of an array where single beads are loaded in microwells. b) Close-up of a single particle in a microwell.

These results demonstrate the unique advantages for performing particle patterning with our device, as the hydrophilic-in-hydrophobic micropatterns allow very efficient removal of excess beads on the Teflon-AF surface by the drag force exerted by the receding droplet meniscus. Moreover, due to the capability of DMF to perform many seeding cycles by simply shuttling droplets over the array many times, virtually all microwells can be filled with single beads.

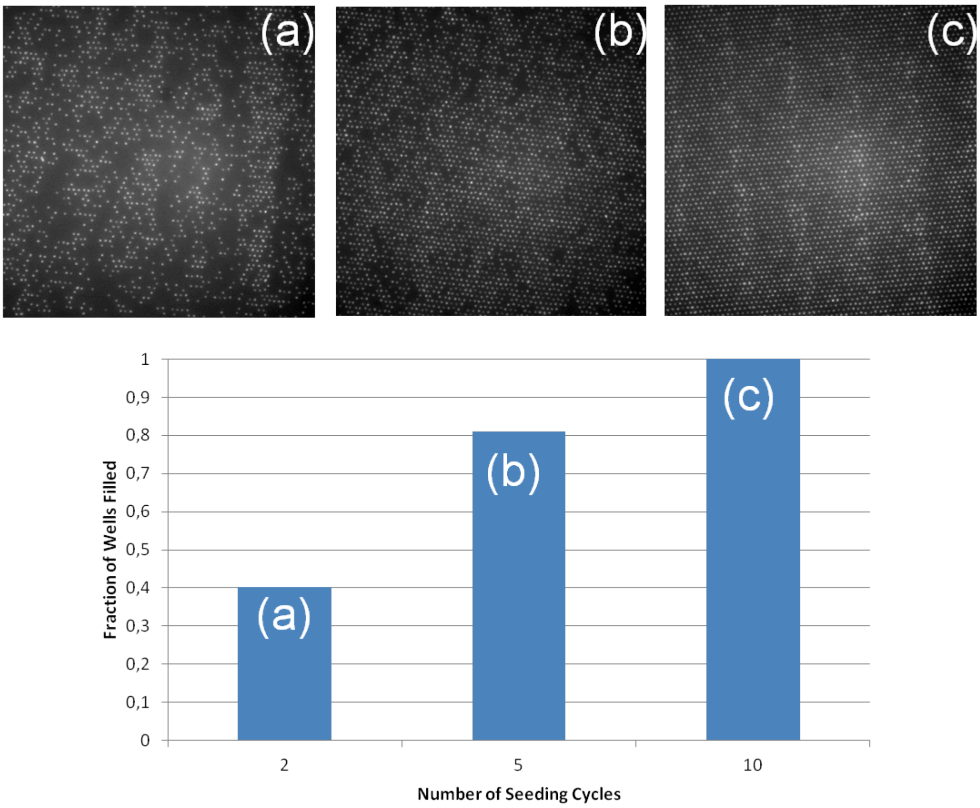

Figure 3: a,b) Fluorescent images of patterned GFP-functionalized magnetic beads on a capture array after 2 seeding cycles (a), 5 seeding cycles (b), and 10 seeding cycles (b). c) Graph demonstrating the increase in loading efficiency with increasing number of seeding cycles.

SUMMARY

DMF was introduced as a novel method for obtaining high-resolution patterns of superparamagnetic beads in femtoliter-sized microwells. Compared to other microfluidic methods, DMF does not require any moving parts or connections to external pumps, and offers very high loading efficiencies (>95%). We anticipate that the unprecedented speed and accuracy with which dense particle arrays are generated with DMF will greatly facilitate detection and readout of magnetic particles in a wide variety of bio-assays.

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CONTACT

Jeroen Lammertyn: +32 16 3 21459 or jeroen.lammertyn@biw.kuleuven.be