A Lab-on-a-Chip using magnetic droplets

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ABSTRACT

We present a chip-based bio-analysis system using the two dimensional (2D) magnetic manipulation of microdroplets. The latter contain magnetic microparticles which serve as force mediators for the droplet actuation. In combination with a changeable field topology over the chip surface, we are able to perform all droplet manipulation steps, like transport, merging, mixing and splitting of droplets on our chip, as presented recently [1]. Based on such a droplet manipulation system, we implemented different bio-analysis protocols such as the on-chip colorimetric detection of biologically active markers and the on-chip purification of DNA. Both procedures show excellent results and demonstrate the potential use of the two-dimensional magnetic droplet manipulation system as a platform for miniaturized bio-analytical systems.

Keywords: Droplets, Magnetic Particles, Bio-analysis, DNA, Microfluidics, Lab-on-a-Chip

1 INTRODUCTION

The miniaturization of bio-analytical processes is an area of vast potential for droplet-based microfluidic systems [2]. Here, small self-contained samples (microdroplets) help to reduce reagent consumption and processing time. In addition, the use of miniature droplets decreases the contact interfaces with the manipulation platform, which minimizes problems of biomolecules adsorption to sidewalls [3]. Recent reports present droplet manipulation systems using a variety of actuation schemes, based on electrostatics [4], acoustics [5], magnetism [6] and electro-wetting [7]. Among those, the magnetic actuation offers the advantage of long range and large force [8]. A further point in favor of the magnetic scheme is the use of paramagnetic particles for the actuation. These particles not only serve as force mediators but also as mobile substrates for biomolecules [9], which permits the implementation of magnetic bead based bio-analytical protocols into the system.

2 SYSTEM SETUP

In our 2D droplet manipulation system the force on the particles inside the droplets is generated via the variable magnetic field over a Printed Circuit Board (PCB) placed in the homogeneous field of permanent magnets (figure 1). A reservoir, sitting on top of the PCB, contains an octamethyl-trisiloxane-based oil that allows aqueous droplets to sink to the reservoir bottom while maintaining their spherical shape. The reservoir bottom is made of a thin (25 μm) Teflon foil whose high hydrophobicity enables a low friction droplet-sliding. For locally defined droplet merging and mixing we immobilize droplets on hydrophilic areas at the surface. These areas of increased droplet adhesion are obtained via a local oxygen plasma treatment of the Teflon. The hydrophilic patterns have a defined shape which facilitates the process of droplet splitting (figure 2). Thus a small droplet loaded with magnetic particles can be passed through a series of immobilized sample and reagent solutions.

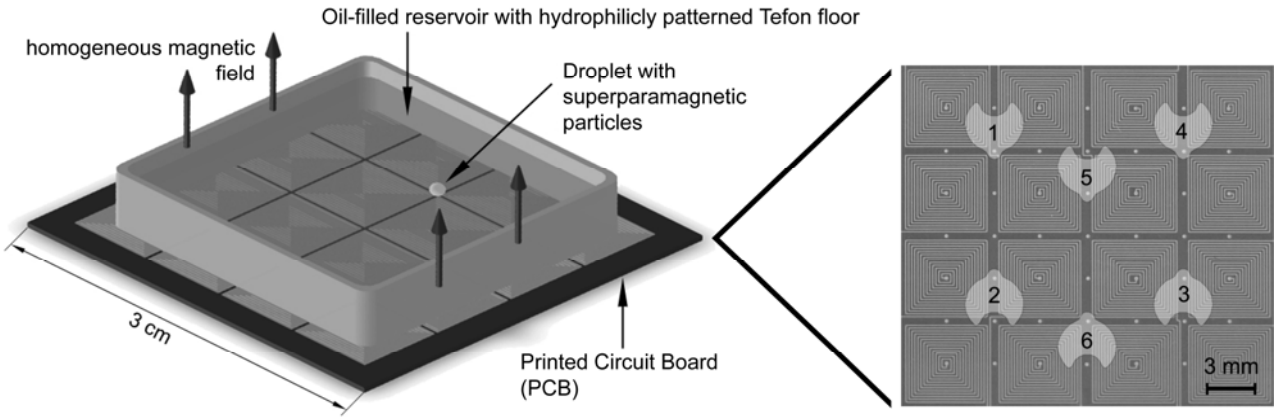

Figure 1: Schematics of the droplet manipulation system, with a top view of the hydrophilic pattern on the reservoir's Teflon bottom (the numbers denote the sequence of using the different positions).

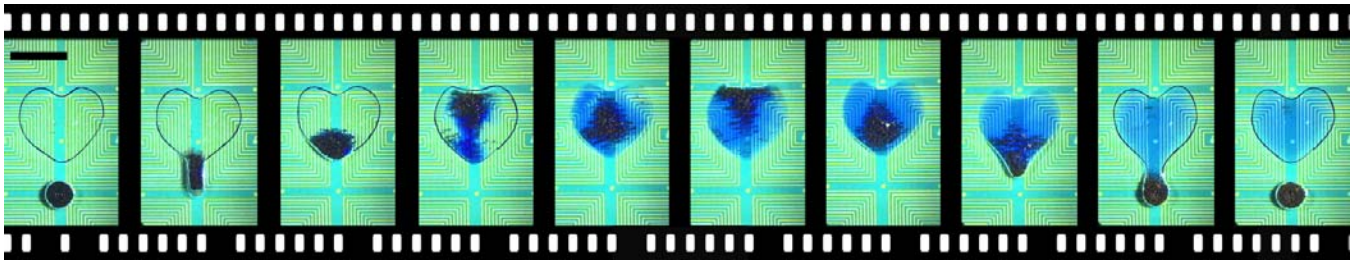

Figure 2: Droplet manipulation sequence. A clear immobilized droplet of 10 µl is merged with a 2 µl droplet carrying magnetic particles and a blue color marker. Both droplets are mixed and separated, leaving the immobilized droplet with a light blue tint. The whole process takes 45 s with durations of 9s for the mixing and 30 s for the splitting. (bar = 3 mm).

3 EXPERIMENTS

We tested our system using two different bio-analytical protocols. In the first case we did not attach any molecules to the particle surface, while in the second case the magnetic particles served as mobile substrate. The magnetic particles we used in our experiments are obtained from the Roche MagNA Pure LC DNA Isolation kit I. They have an average diameter of 6 µm and a hydrophilic silica surface. In our experiments we used sample volumes of 10 µl for the immobilized droplets, and 2 µl and 4 µl of magnetic particles for the colorimetric detection and DNA purification, respectively.

3.1 Colorimetric detection of a bio-active marker in solution

In the first set of experiments we take advantage of the fact that the particles extract a fraction of the sample liquid during the splitting step (see figure 2). Thus a bio-active molecule in solution can be transferred to a second droplet by simply extracting a small droplet of magnetic particles. The transferred molecules can thus be detected or diluted further, if necessary.

We used the colorimetric reaction between the chromogen liquid substrate 2,2'-Azino-bis[3-Ethylbenzthiazoline-6-Sulfonic acid] (ABTS) and Horseradish Peroxidase (HRP) to detect a HRP-labeled antibody. The change in color due to different antibody concentration was registered using a CCD-camera and conventional PC-software. We calculated the color difference value DE (equation 1) with respect to the background. The value DE is determined using the coordinates a, b and L of the CIELAB-color space of droplet (1) and background (0):

$$DE = \sqrt{(a_1 - a_0)^2 + (b_1 - b_0)^2 + (L_1 - L_0)^2} \qquad (1)$$

Using this technique, we measured the color reaction of different concentrations of an HPR-labeled antibody (Rabbit Anti-Mouse Immunoglobulin). The sample concentrations examined in our experiments ranged from 0.6 ng/ml to 130 µg/ml. The dilutions were either prepared outside the chip or obtained directly on-chip. We also examined the effect of multiple stages of on-chip dilution in order to implement an integrated particle washing procedure on the chip.

3.2 On-chip purification of DNA

Our second set of experiments expanded the role of the magnetic particles, as in addition to their role in actuation, they are used as mobile substrates. In these experiments, we attach a molecule to the particle surface and pass it through a series of immobilized droplets.

To test this approach, we used *HinD*III-digested lambda-DNA at different concentrations. The DNA was bound to the silica surface of the particles in a guanidine thiocyanate (GuSCN) binding buffer. The particle-DNA compound is then extracted from the binding site via a separation step and passed through a series of three washing steps. The first washing droplet contains ethanol, water and guanidinium hydrochloride, while the following two hold ethanol and water. The cleaned captured DNA is finally

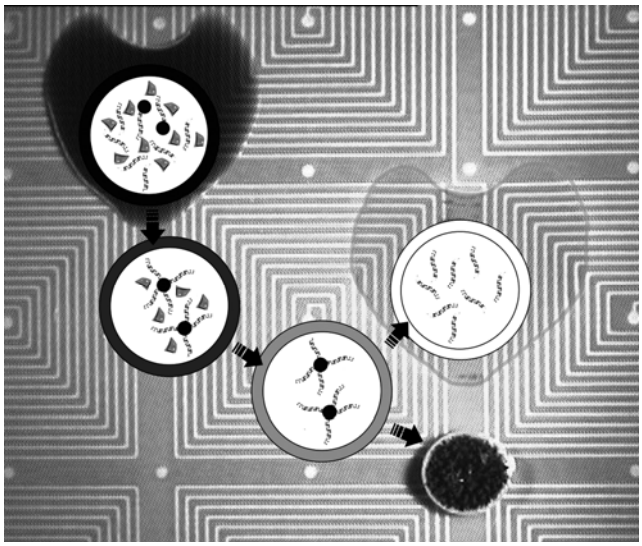

Figure 3: Schematics of the on-chip DNA purification on the background of a photograph of two immobilized droplets on the chip.

transferred to a droplet of low ionic strength and thus eluted from the particle. Figure 3 shows a schematic summary of the DNA purification procedure. After particle extraction, the purified DNA in solution can then detected on- or off-chip using a fluorescent quantification protocol based on the PicoGreen reagent.

For the off-chip detection we extracted a fraction (5 µl) from the droplet of eluted DNA and added 20 µl DI water and an equal volume of the PicoGreen reagent. Due to this additional dilution step before the detection, a fairly high initial DNA concentration was required to match the detection limits of the readout equipment (Cytofluor® series 4000, Perceptive Biosystems). The on-chip detection only required the addition of 10 µl of the PicoGreen reagent to the droplet of eluted DNA. All experiments were repeated at least three times to ensure their reproducibility.

4 RESULTS AND DISCUSSION

Using the two-dimensional magnetic droplet manipulation system, we are able to show the transfer of bioactive molecules in solution from one droplet to another. The color reaction is also function of the concentration of the molecule, since the strength of the blue tint decreases with decreasing immunoglobulin concentration. An example is shown in figure 4, where different concentrations of an enzyme-labeled antibody are transferred to a droplet containing the chromogen.

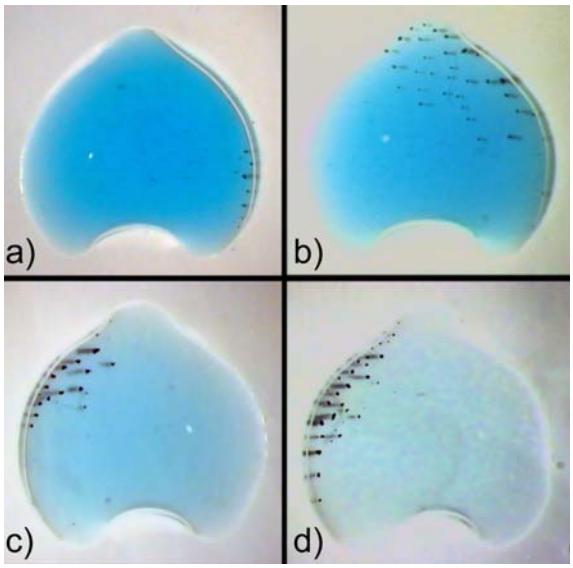

Figure 4: Color reaction in the chromogen solution (10 µl) after 1 min for different concentrations of Rabbit Anti-Mouse Immunoglobulin-HRP:
a) 130 ng/ml, b) 22 ng/ml, c) 3.6 ng/ml, d) 0.6 ng/ml

Figure 5 presents the calculated color difference values in the detection droplets, showing an increase in color intensity with increasing concentration of the secondary antibody. We obtain a logarithmic dependency and a

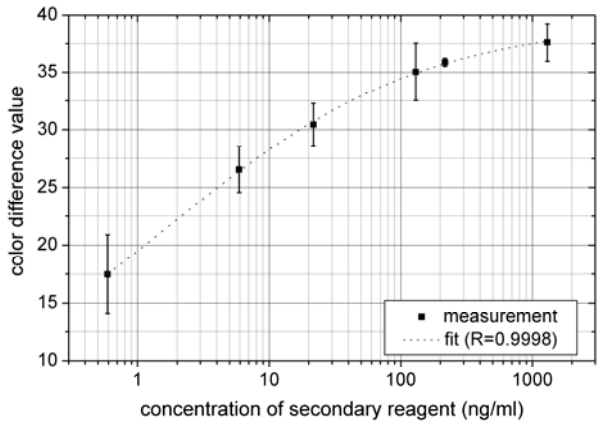

Figure 5: Measurement of the color change with respect to the background for increasing concentrations of Rabbit Anti-Mouse Immunoglobulin-HRP after 1 min of reaction.

detection range of about orders of magnitude (2 logs) with a saturation plateau at higher concentrations, as common for colorimetric detection procedures. We achieve a very high sensitivity, which allows us to measure concentrations as low as 0.6 ng/ml. The dilution stages with small antibody content were achieved via the passage of a transferred sample solution through a second or third droplet containing a Phosphate Buffered Saline (PBS) solution. This successful on-chip dilution of the secondary antibody demonstrated in addition, that the magnetic particles can be washed on-site by passing them through a series of immobilised droplet of PBS. This possibility of on-chip washing enables the particles to remain in the system for continuous droplet processing.

The extended protocol, where DNA is attached to the particles, then washed and eluted, also showed very promising results. The off-chip examination of the capture rates demonstrated a DNA capture between 70 % and 90 %

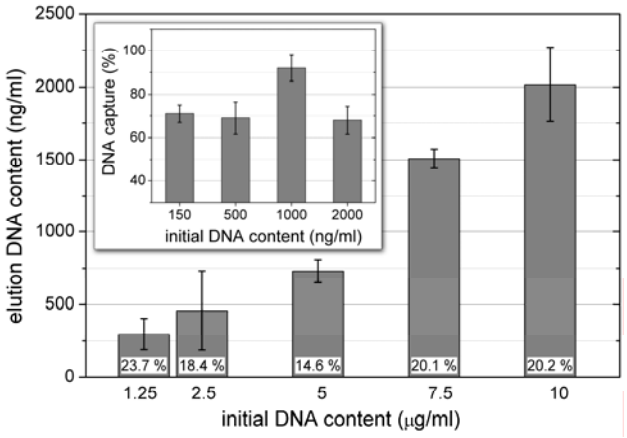

Figure 6: Measured DNA recovery in the elution buffers for off-chip fluorescent detection. The inset shows the capture rates for different DNA concentrations as measured off-chip after the binding step.

with a high reproducibility between experiments. The higher capture efficiency for DNA concentrations in the range of about 1 μg/ml also results in a higher overall recovery after the elution step, as figure 6 shows. The DNA content in the elution buffer, was measured off-chip and ranges from 15 % to 24 %. The high start concentrations are necessary since the fluorescent signal is close to the lower detection limit due to the high dilution necessary for the off-chip quantification. On-chip fluorescent measurements, which do not suffer from increased dilution, indicated a DNA recovery after elution of up to 50 %.

5 CONCLUSION

Our experiments demonstrated the feasibility of using a two-dimensional droplet manipulation system for bio-analytical applications. We could show that with such system, one can transfer small amounts of liquids from one immobilized 10 μl droplet reservoir to another, which allows the detection of concentrations of biomolecules in solution down to a few ng/ml. Another application is the capture of DNA on the magnetic particle surface and its purification. We could recover and detect down to a few μg/ml of a DNA test solution after an elution step on-chip. In both type of applications, we demonstrated the feasibility of the bio-analytical protocols performed in our two-dimensional magnetic droplet manipulation system.

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