Abstract—This paper describes a technique to increase the efficiency of magnetic concentration on an electrowetting-on-dielectric (EWOD)-based droplet (digital) microfluidic platform operated in air, i.e., on dry surface. Key differences in the force scenario for droplet microfluidics vis-à-vis the conventional continuous microfluidic systems are identified to explain the rationale behind the proposed idea. In particular, the weakness of the magnetic force relative to the bead–substrate adhesion and the liquid–air interfacial tension is highlighted, and a new technique to achieve high-efficiency magnetic collection with the assistance of the interfacial force is proposed. An improvement in collection efficiency (e.g., from ∼73% to ∼99%) is observed with the new technique of “meniscus-assisted magnetic bead collection”. In addition, isolation of the magnetic species from a mixed sample of magnetic and nonmagnetic beads is demonstrated. Comparison with other related reports is also presented.

I. INTRODUCTION

HERE HAS BEEN an increasing interest in microfluidic systems over the last couple of decades [1], [2], particularly in “lab-on-a-chip” for numerous biochemical applications such as DNA analysis [3], protein detection [4], and cell-based assays [5]. One important step in the sample preparation of many of these applications is the separation or concentration of the specific cells or molecules of interest from complex mixtures [6], [7]. Various mechanisms, based on magnetic, optical, mechanical, and electrical principles, have been reported [8]. Magnetic concentration and separation on lab-on-a-chip devices have been increasingly popular for their unique advantages over other mechanisms [9]. Unlike electric mechanisms, for instance, magnetic interactions are generally unaffected by surface charges, pH, or ionic concentration. Magnetic manipulation is possible using an external magnet that is not in direct contact with the fluid, not requiring complex structures or electrical circuitry.

Although the native magnetic properties have been explored at times (e.g., magnetic separation for red blood cells [10]), most biological species do not exhibit useful magnetic behavior. The much more common approach is to selectively “label” the species of interest to superparamagnetic beads [also referred to as magnetic beads (MBs)], which respond strongly to magnetic field gradients [11]. The beads’ surface can be suitably modified to achieve specific binding and subsequent isolation of the bound targets such as proteins [12], [13] and cells [14], [15].

Since conventional microfluidic devices are based on continuous flows of liquids through microchannels, most reports of magnetic concentration and separation for microfluidics have been on such a platform. In a typical MB-based immunoassay or cell separation assay (e.g., [12] and [14]), antibody-conjugated MBs (“MB-Abs”) passing through a channel are trapped from flow using an external magnet. Next, the protein or cell sample is flowed through the same channel, where the specific target proteins or cells bind to the trapped MB-Abs. The unbound proteins and cells are subsequently washed away by flowing a wash buffer. A detection antibody, labeled perhaps with a fluorescent tag, may then be flowed, followed by the wash buffer to remove the unbound detection antibodies. The MBs are subsequently released into flow by removing the external magnet to send them to the subsequent steps downstream.

Due to its simple design, low power consumption, and reprogrammable fluid paths, droplet-based or digital microfluidics driven by electrowetting-on-dielectric (EWOD) [2], [16], [17] is an attractive platform technology to develop microfluidic devices and systems on for many applications. Unlike continuous flows through channels, fluids are handled in the form of droplets (hence, droplet or digital microfluidics) driven by electrically controlling the wetting property of the surface (hence, electrowetting) typically of a dielectric in recent years (hence, EWOD, as opposed to the regular electrowetting that had been known on metal surfaces for many years). Although some biochemical applications of EWOD have been shown [18], [19], many are yet to be accomplished. Target separation is one of the key steps in making EWOD more powerful as a lab-on-a-chip platform. Mechanisms explored for target separation and concentration inside the droplet include electrophoretic [20], dielectrophoretic [21], [22], and magnetic [23], [24].

Magnetic concentration using just a permanent external magnet is an attractive option due to its simplicity. Analogous to the steps in continuous microfluidics, concentration steps for digital microfluidics would require cycles of adding sample or wash-buffer droplets and removing the unbound proteins and labeled Ab as outgoing droplets. The collection efficiency of MBs in these steps eventually determines the sensitivity of
detection and is therefore of critical importance. The next section highlights the different force scenario in droplet microfluidics vis-à-vis continuous microfluidics, which exposes a fundamental difference in the way the MBs are concentrated and forms the rationale for the proposed technique.

On EWOD droplet microfluidic platform, collection of MBs with good efficiency has so far been limited to oil environment [25]–[27]. When the device is immersed in oil [28]–[30] rather than dry in air [16], [31], [32], a thin layer of oil present between the hydrophobic device surface and the aqueous droplet [33] greatly reduces the resistance against droplet sliding, making most of the basic EWOD operations much less challenging. The thin oil layer also separates the MBs from the device surface, preventing adhesion of MBs on the surfaces and easing their collection. Despite the conveniences, there have been some concerns, as listed in the following, regarding the use of oil, particularly in biological applications.

1) Even though oil prevents adsorption of proteins onto the hydrophobic surface, there is the possibility of protein adsorption and entrapment (emulsification) at the water–oil interface [34]. This may lead to the loss of proteins that could adversely affect the assay and/or detection, besides contamination of the surrounding medium.

2) If sample droplets need to be dried on chip, such as the multiplexed MALDI-MS on EWOD platform [35], immersing in silicone oil is incompatible. Not only is drying difficult for droplets in oil, but a trace of silicone oil on the dried surface can also interfere with detection signals.

3) If the application requires certain events to be performed directly on the device surface, such as hybridization of nucleotides on the surface or reading with electrochemical sensors patterned on the surface, the thin layer of oil could hinder the performance.

4) The surrounding silicone oil may restrict the exchange of gases between the droplets and the atmosphere, which may be unfavorable for biological cells in the droplet. In particular, it could lead to a buildup of carbon dioxide in the droplets, which may suffocate the cells.

5) Ensuring that the oil does not leak makes the packaging of the device much more challenging.

6) The devices developed for in-air operations, which are far more demanding, work for both in air and oil but not vice versa. General-purpose, or ideally universal, EWOD devices should function without having to immerse in or treat with oil.

In this paper, we consider the case where the surrounding medium is air. Comparison with other reports [24], [36] that have obtained good magnetic concentration on EWOD in air without using the technique reported in this paper will be provided later in Section IV.

II. THEORY

A. Forces on an MB in an Aqueous Droplet in Air on an EWOD Surface

The interfacial force at the liquid menisci, while absent in continuous flows, is a major force at play in droplet microfluidics. While the advancing meniscus of a droplet picks up particles on the dry surface into the droplet [37], our interest is with the receding meniscus, which keeps the particles inside the moving droplet instead of leaving them behind on the dry surface. As will be discussed hereinafter, the force with which the receding meniscus pulls the MB away from the surface turns out to be much stronger than other forces including magnetic.

Consider an MB located at the receding meniscus of the droplet, in the presence of an external magnet. Fig. 1 shows the forces of primary interest in this case: the surface adhesion force viz. van der Waals (vdW) force \(F_A\), the magnetic force \(F_{mag}\), and the liquid interfacial tension \(F_Y\). Other forces on the MB like gravity, viscous drag, and buoyancy turn out to be much smaller than these three.

The vdW force between sphere 1 and surface 2 in medium 3, as shown in Fig. 1, is given by:

\[
F_A = \frac{A_{132} R_{MB}}{6H^2}, \quad H \ll R_{MB}
\]

where \(A_{132}\) is Hamaker constant and \(H\) is separation. Typically, \(H\) is assumed to be 0.5–1 nm, and \(A_{132}\) can be estimated based on individual material properties [37], [38]. Values for the Hamaker constant in vacuum for polystyrene \((A_{11} = 7.8 \times 10^{-20} J)\) and protein \((A_{11} = 7.3 \times 10^{-20} J)\), used to calculate the aforementioned three-component Hamaker constant, are quite similar [38]. Based on these, the vdW force for a polystyrene- or protein-coated bead over a typical EWOD surface (Teflon or Cytop) in water is estimated to be \(\sim 10^{-9} - 10^{-10}\) N.

The magnetic force acting on a magnetic dipole inside a magnetic field is given by:

\[
F_{mag} = (m \cdot \nabla)B = \frac{1}{2} V \mu_0 \nabla B^2
\]
The magnetic flux density near a cylindrical NdFeB magnet, 12.7 mm thick and 12.7 mm in diameter (half cross section shown). (b) $z$-component of the magnetic force acting on a 1-$\mu$m-radius MB placed in this magnetic field as a function of distance $z$ from the magnet’s face and distance $r$ from the magnet’s axis of rotation. It should be noted that the order of magnitude of $F_{mag,z}$ is $10^{-10} - 10^{-12}$ N.

The order of magnitude of $F_{\gamma,z}$ is $10^{-7} - 10^{-8}$ N. where $V = $ volume of the magnetic particle, $\chi = $ magnetic susceptibility, and $B = $ magnetic flux density.

A Finite Element Method Magnetics (FEMM) software simulation was used to estimate the magnetic flux density and its gradient that is $\sim$1 mm from the surface of an NdFeB permanent magnet [Fig. 2(a)]. Assuming the magnetic susceptibility of a polystyrene microsphere with iron oxide core as $\sim$0.1 and (over)estimating the magnetic volume to be the entire MB volume, the magnetic force on it turns out to be $\sim 10^{-10}$ to $10^{-12}$ N [Fig. 2(b)].

Last, the force due to interfacial tension and its $z$-component are given by

$$F_{\gamma} = 2\pi R MB \gamma_{lg} \sin \phi \sin(\phi - \alpha)$$  (3)

$$F_{\gamma,z} = F_{\gamma} \cos \theta$$  (4)

where $\gamma_{lg} =$ liquid-vapor surface tension, $\alpha =$ contact angle on the MB surface, $\theta =$ contact angle of the surface, and $\phi =$ angle determined by the immersion of the MB into the liquid, as shown in Fig. 3 (inset).

As the receding meniscus sweeps across the MB, the vertical component of the liquid interfacial force $F_{\gamma,z}$ pulling the MB away from the surface into the droplet exceeds the surface-adhering forces. Fig. 3 shows the maximum values ($F_{\gamma,z,max}$) of $F_{\gamma,z}$ (maximized over $\phi$) generated using MATLAB for varying values of $\alpha$ assuming $\theta = 110^\circ$ (typical value on EWOD surface). Typical values of this force range from $\sim 10^{-7}$ to $10^{-8}$ N, which are 2–3 orders of magnitude higher than either the magnetic or the vdW force, implying that the interfacial force will dominate.

B. Proposed Idea: Meniscus-Assisted MB Collection and Separation

By keeping in mind the force scenario described earlier, the new technique of “meniscus-assisted MB collection” is proposed, which is extended from [23]. Fig. 4 shows the concept of the proposed technique. When a magnet is introduced, the MBs suspended in the droplet move toward it [Fig. 4(a-1)], but many of those on the surface do not. Magnetic force is ineffective in moving the MBs that are already in contact with the device surface (by $F_{mag,z}$ or gravity) against the surface adhesion force. As a result, no significant region of the droplet becomes MB free. Therefore, when the droplet is cut as part of the wash step, many MBs will be lost in the outgoing droplet [Fig. 4(a-2)], making the wash steps inefficient. Acknowledging that meniscus crossing is unavoidable for droplet microfluidics, we instead propose to utilize the powerful liquid interfacial force to sweep the particles off the surface into the droplet, so that the magnetic force will move the suspended particles to one side. To achieve this, the droplet is moved toward the left by EWOD [Fig. 4(b-1)]. As the meniscus moves, it sweeps the MBs on the surface back into suspension, so that they can be collected by the magnet [Fig. 4(b-1), inset]. After all the MBs have been swept up by the receding meniscus [Fig. 4(b-2)], the droplet is moved back to the right of the magnet so that the MBs are collected on the left side of the droplet [Fig. 4(b-3)]. The droplet is subsequently split [Fig. 4(b-4)]
Fig. 4. Schematic description of the meniscus-assisted MB collection technique. (a) Existing practice, where a magnet attracts the MBs to one side in a stationary droplet before splitting it. (a-1) When a magnet is introduced over the droplet, some MBs move toward the magnet, but other MBs on the surface do not move effectively. (a-2) As a result, many MBs are left in the droplet that is meant to be depleted of MBs. To overcome the problem, the magnetic attraction should be performed quickly before the MBs settle on the surface [24]. (b) Proposed procedure, where the droplet follows a prescribed path relative to the magnet during the magnetic attraction, before splitting the droplet. (b-1) As the droplet is moved to the left, (inset) the receding meniscus sweeps the MBs off the surface and into suspension, allowing the magnetic force to collect the now resuspended MBs. (b-2) Droplet is moved leftward, collecting all the MBs to under the magnet. (b-3) When the droplet is moved back to the right of the magnet, the MBs are concentrated near the receding meniscus. (b-4) With the MBs collected on the left side, the droplet is split. Most of the MBs are in the (left) “collected” droplet, while the (right) “depleted” droplet is nearly MB free. Compare with (a-2).

Fig. 5. Schematic diagram to show how the proposed technique can be used to increase the relative concentration of MBs in a droplet containing a mixture of (dark circles) MBs and (light circles) non-MBs. (a) When a magnet is introduced over the device, many MBs move toward the magnet. However, the non-MBs and some MBs, particularly those touching the surface, do not. (b) Droplet is then moved leftward. (Inset) As the beads are swept off the surface and resuspended by the receding meniscus, the MBs are collected by the magnetic force, but the non-MBs are not. (c) Droplet is moved further to the left until all the beads on the surface are swept up by the receding meniscus. (d) Droplet is moved back to the right of the magnet. While the MBs are collected on the left side, the non-MBs distribute uniformly across the droplet. (e) Droplet is split using EWOD, so that (f) most of the MBs are in the left (“collected”) droplet, while the non-MBs are distributed roughly in proportion to volumes. (f) By adding buffer and repeating steps (b)–(e), the non-MBs can be serially diluted to increase the purity of MBs.

The proposed technique can be further used to purge out nonmagnetic species in a droplet containing a mixture of magnetic and nonmagnetic particles. As shown in Fig. 5, a droplet containing both MBs and non-MBs is taken through the steps (a–e) of meniscus-assisted MB collection. As the droplet is moved leftward, [Fig. 5(b) and (c)], all the beads on the surface are swept into suspension by the receding meniscus. While the MBs get collected by the magnet [Fig. 5(c)], the non-MBs distribute across the droplet. The droplet is moved back to the right of the magnet [Fig. 5(d)] and split [Fig. 5(e)]. Most of the MBs are retained in the collected droplet, while the
non-MBs are distributed between the collected and depleted droplets in proportion to their volumes. The depleted droplet can be removed, and after adding a buffer droplet to the collected droplet [Fig. 5(f)], the same steps can be repeated. With each iteration, the non-MBs are removed in the form of “depleted” droplets (depleted of MBs) while retaining most of the MBs in the “collected” droplet (where MBs are collected), thus decreasing the concentration of non-MBs against MBs.

It is worth mentioning that the focus of our idea is on MBs at the receding meniscus, which is typically not subject to any voltage (since the electrode underneath is grounded). Therefore, we do not expect electromechanical forces to be a factor in particle detachment at the receding meniscus. Electromechanical agitation at the advancing meniscus, where the voltage is applied, is plausible. However, in our experiments, most of the EWOD voltage drop occurs across the dielectric layer (particularly since our dielectric and hydrophobic layers are relatively thick compared, for example, to that in [37] and [39]), and not across the droplet, even at 1 kHz frequency. For different device geometry and material properties, though, this phenomenon could indeed be more prominent, as noted in [39].

III. MATERIALS AND METHODS

A. Device Fabrication and Droplet Actuation

Lithographic thin-film microfabrication processes were used to fabricate the parallel-plate EWOD device (Fig. 1). Square driving electrodes of 1–1.2 mm width were defined from an indium tin oxide (ITO) (140 nm) layer over a 700 μm-thick glass substrate (TechGophers Corporation). Cr/Au (∼15/150 nm) was deposited and patterned to define the contact pads and electrode labels for easier visualization. Next, a silicon nitride layer (∼1000 nm) was deposited using plasma-enhanced chemical vapor deposition (PECVD) and patterned to define the dielectric layer. A Cytop (Asahi, Inc.) layer (∼1000 nm) was spin coated on top and cured at 200 ◦C to make the surface hydrophobic. Some ITO-coated (140 nm) 1.1-mm-thick glass substrates (Delta Technologies, Inc.) were used to prepare the “reference” substrate. A thinner PECVD Si₃N₄ layer (∼100 nm) was deposited and patterned on it to expose the ITO for electrical ground connection, followed by Cytop (∼100 nm) deposition. Double-sided tape (∼0.1 mm thick; 3M, Inc.) was used as the spacer between the two substrates sandwiching the droplet(s).

Droplet actuation was achieved by applying voltage typically of ∼70 V ac at 1 kHz to EWOD electrodes. Electronic control for the actuation sequence was controlled using LabVIEW with the help of a digital I/O device. Magnetic force was provided using a cylindrical permanent magnet (NdFeB, 12.7 mm thick and 12.7 mm in diameter) placed on top of the EWOD device (Fig. 1).

B. Reagents and Samples Used

For MBs, Dynal-Invitrogen’s Dynabeads were used in two sizes—4.5 μm (M450 epoxy) and 1.0 μm (MyOne Streptavidin T1) in diameters. Dynabeads have a ferromagnetic core surrounded by a polystyrene shell, often coated with proteins like streptavidin or antibodies, and are, by far, the most commonly used MBs [9], [40] in biological applications like immunoassays (e.g., [41]) and cell separation (e.g., [42]). For nonmagnetic fluorescent beads (FBs), Nile-red fluorescent (535/575 nm) carboxylate-modified polystyrene microspheres from Molecular Probes, Inc. (now Invitrogen, Inc.) were used in two sizes—2.0 μm (Fluospheres F8825) and 5.3 μm (Interfacial Dynamics 2-FN-5000) in diameters. To eliminate any other additives present in the beads’ stock solutions, all beads were washed twice and resuspended in phosphate-buffered saline (PBS) for EWOD experiments.

C. Visualization and Image Capture

The EWOD device, with droplet(s) sandwiched between substrates, was mounted on an inverted fluorescence microscope (Nikon TE-2000 U) for visualization. A video camera (KR-222, Panasonic) was used to capture the droplet actuation movies, while bright-field and fluorescence still images were taken using a cooled charge-coupled device (CCD) camera (Coolsnap EZ, Photometrics).

Particle counting was performed using the images taken on-chip by the cooled CCD camera. Before taking these images, the droplet was shuttled across a few electrodes without the magnet in place to break up (at least some of) the clusters, particularly for MBs. The concentration of particles was also kept small enough (< 10⁴/μL) to keep the direct counting manageable, instead of transferring it to a different counting device such as a hemocytometer [24], which could add to the error.

D. Image Analysis Using ImageJ

Image analysis was performed using ImageJ software to determine the droplet volumes and particle counts. Images just after each droplet cut were analyzed to find the area of each droplet, and the volume was estimated based on the area and the known spacer thickness. Images from the cooled CCD camera were analyzed for three cases of bead samples. Cases 1 and 2 contained only MBs (4.5 and 1.0 μm, respectively), while Case 3 contained a mixture of MBs (4.5 μm) and FBs (2.0 and 5.3 μm). The cases are described in detail in the next section.

To count the 4.5-μm MBs in Case 1, bright-field images were used. By manual inspection, the intensity threshold and size in pixel squared for each MB singlet, doublet, triplet, and quadruplet were estimated. Based on these values, counts were done using the automated particle counting function (“Analyze Particles”) in ImageJ. Manual inspection was performed to add any remaining clusters and/or beads adjacent to the meniscus and other dark features and also to eliminate any spurious MBs picked up by the program. The 1-μm MBs used in Case 2 are harder to visualize because they are barely visible at the typical magnification (1–4×) used for droplet visualization. At higher magnifications, the field of view is too small to see the entire droplet or significant regions of it at a time. In this case, therefore, images of droplets taken at a higher magnification (10×) were stitched together and used for counting. In cases where automated counting seemed unable to identify
the MBs correctly, manual counting was performed. For Case 3, the intensity threshold and size (in pixel squared) of FBs were determined by manual inspection (as in Case 1). Unlike MBs, FBs are not prone to forming clusters. Therefore, only singlets and doublets were counted using the ImageJ function. Manual inspection was then performed to add or subtract any unaccounted or incorrectly picked FBs.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

In order to demonstrate the proposed idea described in Section II, two sets of experiments were performed. First, the difference in collection efficiency between without and with meniscus sweep was highlighted for MB concentration, testing two different sizes of MBs (as shown in Fig. 4). Second, MB separation was demonstrated using meniscus-assisted serial purification, whereby nonmagnetic FBs were depleted while MBs were retained (as shown in Fig. 5). In all experiments, a strong permanent magnet described earlier was used to maximize the magnetic force in the presence of surface and interfacial forces that were stronger. Similar results can be expected with smaller magnets, where the interfacial and surface forces will dominate over the magnetic force even more.

A. MB Collection Without and With Meniscus Sweep

Case 1: MB Collection With 4.5-μm MBs: The effect of meniscus assistance on collection efficiency was demonstrated using 4.5-μm MBs by performing MB collection without (Case 1a) and with (Case 1b) the proposed meniscus assistance.

Case 1a—Without Meniscus Assistance: Fig. 6 shows the case of MB concentration without employing the meniscus sweep. An NdFeB magnet is placed over the EWOD device to the left of the droplet containing 4.5-μm MBs [Fig. 6(a)]. Many of the MBs move toward the magnet due to the magnetic force. However, this force is ineffective in moving the MBs that are touching the device surface, as per the discussion in Section II. After about 30 s, therefore, when the droplet is cut [Fig. 6(b) and (c)], not all the MBs are collected in the left-side (“collected”) droplet [Fig. 6(d)]. Fig. 6(e) shows a clearer image of the collected droplet with a magnified view. More importantly, many MBs are (undesirably) present in the right-side (“depleted”) droplet, as seen in Fig. 6(f), leading to a moderate collection efficiency (∼73%).

Case 1b—With Meniscus Assistance: Next, MB collection was performed using the proposed technique of meniscus-assisted MB collection described before (see Fig. 4). A fresh droplet from the same sample was used. As in Case 1a, the NdFeB magnet is placed over the EWOD device to the left of the MB-containing droplet [Fig. 7(a)]. The droplet is then moved under the magnet and back using EWOD. In the process, the receding meniscus sweeps the MBs off the surface back into solution, allowing them to be attracted leftward by the magnet [Fig. 7(b)] (this sweep sequence typically takes less than 10 s). The droplet is subsequently split by EWOD [Fig. 7(c)], with most of the MBs (∼99%) collected in the left-side (“collected”) droplet [Fig. 7(d)]. Fig. 7(e) and (f) shows the collected droplet, which has a greater fraction of MBs than in Case 1a, despite a smaller volume fraction. The difference in the two cases is more apparent when we compare Figs. 6(f) and 7(f). The right-side (depleted) droplet is nearly MB free in Fig. 7(f), while the one in Fig. 6(f) still contains many MBs. For the case of 4.5-μm MBs, therefore, one can see that meniscus sweeping helps achieve a very high collection efficiency (∼99%), which is a significant improvement over the no-sweep Case 1a.
Fig. 7. (Case 1b) Collection efficiency is drastically improved with meniscus assistance for 4.5-μm MBs. Images (a)–(d) depict the actuation sequence, while images (e) and (f) show the collected and depleted droplets, respectively. (a) Magnet is introduced near the droplet containing 4.5-μm MBs. Some MBs move to the left, but once they touch a surface, they can no longer be pulled by the magnet. (b) Droplet is moved under the magnet and back out, so that the meniscus sweeps the MBs into the solution (see Fig. 4). After the meniscus sweep, the MBs are mostly collected to the left by the magnetic force. (c) Droplet is then cut by EWOD. The MBs can be seen collected at the left meniscus, just before the cut. (d) Left-side (collected) droplet contains most of the MBs. (e) Collected droplet, magnified to show the MBs more clearly. (f) Depleted droplet contains far fewer MBs than Case 1a, indicating a much improved collection efficiency. The cut droplets are shuttled back and forth without magnet to spread the MBs to help in the image analysis.

Fig. 8. (Case 2a) Without meniscus assistance, only moderate collection efficiency is achieved for 1-μm MBs. Images (a)–(d) describe the actuation sequence, while images (e) and (f) show the collected and depleted droplets, respectively. (a) Magnet is introduced near the droplet containing 1-μm MBs. (b)–(c) After ~30 s, the droplet is cut by EWOD with the magnet in place. (d) After the cut, the left-side (collected) droplet does contain majority of the MBs. (e) Collected droplet, magnified to show the MBs more clearly. (f) Depleted droplet, which should ideally be MB free, contains many MBs. The MBs are clearly visible only in the magnified photographs due to the smaller size.

Case 2: MB Collection With 1.0-μm MBs: In order to demonstrate its applicability to the typical range of MB sizes (1–5-μm diameter), similar experiments as Case 1 were performed for smaller (1.0-μm-diameter) MBs, i.e., MB collection without and with meniscus assistance.

Case 2a—Without Meniscus Assistance: Fig. 8 shows the case for 1.0-μm MBs collected without meniscus assistance. Following the same steps as that in Case 1a, similar collection efficiency is observed. The magnet is introduced to the left [Fig. 8(a)]. Although some MBs move, the magnetic force is ineffective in pulling the MBs that touch the surface. As in Case 1a, when the droplet is cut [Fig. 8(b) and (c)], a majority of MBs are collected in the left-side (collected) droplet. However, many MBs are left in the right-side (depleted) droplet. Fig. 8(e)
Fig. 9. (Case 2b) High collection efficiency using meniscus assistance for 1-μm MBs. Images (a)–(d) describe the actuation sequence, while images (e) and (f) show the collected and depleted droplets, respectively. (a) (Left) Magnet is introduced. (b) Droplet is moved under the magnet and back out. The meniscus-swept MBs are collected at the left meniscus. (c) Droplet is cut by EWOD. (d) Left-side (collected) droplet contains most of the MBs. (e) Collected droplet. The MBs can be seen in the magnified view. (f) Depleted droplet. Very few MBs can be seen, indicating high collection efficiency in this case similar to Case 1b. The MBs are clearly visible only in the magnified photographs here due to their smaller size.

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<th># of MBs in collected droplet: C</th>
<th># of MBs in depleted droplet: D</th>
<th>Collection efficiency: #C / (#C+D)</th>
<th>Volume fraction of collected droplet: Vol C / Vol (C+D)</th>
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<td>1818</td>
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Thus, for 1.0-μm MBs as well, meniscus sweeping helps achieve a very high and significantly improved collection efficiency (> 98%) compared to the no-sweep Case 2a.

B. Summary of Results for Cases 1 and 2

The image analysis results for all the four cases described earlier are summarized in Table I (see Section III for details of the image analysis). For all cases, an EWOD device with 1.2-mm × 1.2-mm electrode arrays was used, with an initial droplet volume of ~650 nL. Because significant evaporation occurred over the course of setup, experiment, and image capture, all the data are presented in the form of number of MBs and volume fraction, rather than concentration and absolute volume. Images used for particle counting were taken after moving the droplet over a few electrodes without the magnet in place, so that the MBs were somewhat spread on the surface. MB counting and area calculation were performed using ImageJ software. For both Cases 1 and 2, the number of MBs in the collected droplet was higher in the case of b (i.e., with the meniscus sweep) despite a smaller volume fraction of the collected droplet in this case. It should be pointed out that well below 1% of the particles were left behind after the receding meniscus across the surface. On the few occasions that it was observed, surface heterogeneities (e.g., defects in hydrophobic layer, dirt, etc.) were usually responsible.
Fig. 10. (Case 3) Image sequences showing three successive cycles of meniscus-assisted serial purification steps. MBs (4.5 μm) mixed with nonmagnetic FBs of two sizes (2.0 and 5.3 μm) suspended in PBS are used. Each cycle consists of a meniscus sweep, followed by droplet cut, and buffer addition, performed on the same initial droplet at different locations. (a)–(d) Sweep-and-cut 1: (a) Magnet is introduced at left of the initial droplet. (b) Droplet moved under magnet and back to sweep the MBs with the meniscus and collect them to the left with the magnet. (c) Droplet is cut, with most of the MBs on the left side of the droplet. (d) After the cut, the (left) collected droplet contains most of the MBs, while the FBs are distributed across both the collected and depleted (not seen) droplets. (e) A buffer droplet is added to the collected droplet from (d), and the combined droplet is moved to a different location. (f)–(i) Sweep-and-cut 2: Steps corresponding to (b)–(e). (j)–(l) Sweep-and-cut 3: Steps corresponding to (b)–(d).

The MBs swept up by the meniscus enter the liquid droplet along with the fluidic movement within the droplet. Since the liquid recirculates within the moving droplet in a 3-D flow pattern [43], the fluid packets near the meniscus not only travel toward the opposite device surface but also flow into the body of the droplet, dragging the MBs with them. We observed that practically all of the MBs that swept off the device surface and accumulated near the receding meniscus are already inside the liquid droplet rather than at the meniscus. Even if some MBs were stuck at the meniscus, they would soon get dragged into the liquid with the flow from the meniscus into the droplet, before being collected magnetically.

It should be noted that the MBs used in these experiments have hydrophobic surfaces, as per the product information from the manufacturers [44], [45] (the exact contact angle with water is not available). According to Fig. 3, the interfacial force would be stronger for more hydrophilic particles (such as glass microbeads used in [37], and some other commercially available MBs), suggesting that the meniscus-assisted technique will continue to be effective for their collection as well. The interfacial force is expected to be slightly lower for beads that are more hydrophobic (e.g., the “superhydrophobic” beads used in [37]), although Fig. 3 suggests that it would be on the same order of magnitude. The relatively greater tendency of superhydrophobic beads to stay adsorbed on the droplet meniscus [37] could also limit collection efficiency. However, superhydrophobic beads are extremely rare in use and hardly available commercially (in fact, [37] prepared them in-house “due to the difficulty in finding superhydrophobic spherical particles”). As such, the case of superhydrophobic beads has not been considered in detail here.

C. Comparison to Other Reports not Using Meniscus Assistance

It should be mentioned that MB concentration has been reported without meniscus assistance [24], [36], where the collection efficiency was significantly higher than that in Cases 1a and 2a. However, there are some important differences in the experimental procedures. Wang et al. [24] reported much higher collection efficiency (> 91%) than Cases 1a and 2a, even without meniscus sweep. Acknowledging that “particle adhesion becomes prominent as the magnetic particle solution stays longer on the surface”, which would lower the collection efficiency primarily because the MBs settle down on the surface, they reduced the time between sample loading and cutting execution to “less than a minute”. However, many practical applications may require much longer duration between these
steps. A decrease in collection efficiency was also reported for smaller magnetic particles, since the adhesion force relative to the magnetic force becomes more dominant for them. As a more generalized scenario, therefore, we did not impose such special conditions for the experiments in this paper. There was typically a few (2–10) minutes’ gap between sample loading and droplet actuation. We also show that the high collection efficiency does not decrease when MB size is reduced from 4.5 to 1 μm. Using the interfacial force to overcome surface adhesion, therefore, we mitigate the constraints of execution time and particle size for high collection efficiency (∼99%, as described in [24]).
Another important difference of our test conditions from [24] and [36] is that the beads were carefully washed prior to experiments. Most MBs used for biological applications are supplied in stock solutions containing additives for stability and long-term storage, which are supposed to be washed off prior to use, as per protocol. Additives and other constituents in the medium, which are required at times for actuation of biological samples, may cover the beads’ surface, affecting the surface adhesion $F_A$ and leading to a different force scenario that does not require the meniscus assistance. To be conservative and more general, this paper deals with only the purely physical method of meniscus sweeping without entailing chemical addition and any associated undesirable side effects. The force estimates and results presented here are based on the washed beads suspended in buffer, with presumably little or no surface coverage from the constituents of the medium.

D. MB Separation Using Meniscus-Assisted Serial Purification (Case 3)

Next, it is demonstrated that the MBs (or magnetically labeled targets) in a droplet containing a mixture of magnetic and nonmagnetic species can be separated by meniscus-assisted serial purification. This is demonstrated using $4.5$-μm MBs along with nonmagnetic FBs of two sizes—2.0 and 5.3 μm, suspended in PBS. Pure PBS is used as the dilution buffer for each serial purification step. An EWOD device with 1-mm × 1-mm electrodes array was used for these experiments. The sequence of EWOD actuations performed is shown in Fig. 10. Bright-field and fluorescence images at each stage are shown in Fig. 11.

Case 3—Serial Purification: A PBS droplet containing both MBs and FBs (initial volume ∼650 nL) is pipetted onto the device [Fig. 10(a)], along with two droplets of PBS (∼1000 nL each) at separate locations (not shown). Bright-field and fluorescence images are taken for the initial droplet (Fig. 11(a) and (b), respectively). Next, the magnet is introduced, and the droplet is taken through the steps of Fig. 10(a)–(d) (“sweep-and-cut 1”), as in Fig. 5. Bright-field and fluorescence images of the left (collected) and right (depleted) droplets are taken (Fig. 11(c)–(f), respectively), after which a buffer droplet is merged with the collected droplet [as in Fig. 5(e)]. The combined droplet is then taken to a different location [Fig. 10(e)] where steps of Fig. 10(e)–(h) are performed (“sweep-and-cut 2”), and images are taken for the collected and depleted droplets (Fig. 11(g)–(j), respectively). A second buffer droplet is merged with this collected droplet, and the sequence of steps is repeated at a different location.

Table II presents the summary of results for Case 3. Since the droplet volume changes due to evaporation over the course of setup, experiment, and image capture, the data are presented in the form of number of beads and volume fractions, instead of concentration and absolute volumes, as in Cases 1 and 2. It can be seen that the number of FBs steadily falls with each purification cycle, roughly in proportion to the droplet volume fraction. From the initial droplet to the collected droplet after sweep-and-cut 3, the number of remaining FBs drops to below 10%.

While the FBs are relatively easy to count using the fluorescence images, the only way to count the MBs is using the bright-field images. This is made difficult due to the presence of FBs, which show up in these bright-field images as well. As such, MB counting was performed for one case (collected droplet of “After sweep-and-cut 3”), since this has the fewest FBs and assumed to be the same (∼475) for all collected droplets. This is a reasonable approximation based on the results of Case 1b, where ∼99% of MBs are retained using the technique. The trend for the decreasing FB:MB ratio is shown in Fig. 12 based on these calculations.

V. CONCLUSION

This paper has highlighted the relative dominance of the liquid interfacial force in droplet microfluidic systems, such as an EWOD device, and the challenge that such dominance
posed for magnetic concentration. Based on the force scenario, we proposed to use the interfacial force at the droplet meniscus to assist the magnetic collection. Significant improvement in collection efficiency (from ~73% to ~99%) was observed using the proposed technique. We also demonstrated the use of the technique to purify the MBs by washing the non-MBs off through serial purification while retaining the MBs in the main droplet. The MBs are often used as specific labels to concentrate and separate on EWOD, making EWOD-MBs is directly proportional to the effectiveness of this process.

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