

Continuous-flow ferrohydrodynamic sorting of particles and cells in microfluidic devices

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Abstract A new sorting scheme based on ferrofluid hydrodynamics (ferrohydrodynamics) was used to separate mixtures of particles and live cells simultaneously. Two species of cells, including *Escherichia coli* and *Saccharomyces cerevisiae*, as well as fluorescent polystyrene microparticles were studied for their sorting throughput and efficiency. Ferrofluids are stable magnetic nanoparticles suspensions. Under external magnetic field gradients, magnetic buoyancy forces exerted on particles and cells lead to size-dependent deflections from their laminar flow paths and result in spatial separation. We report the design, modeling, fabrication and characterization of the sorting device. This scheme is simple, low-cost and label-free compared to other existing techniques.

Keywords Cell sorting · Ferrohydrodynamics · Continuous-flow · Ferrofluid · Microfluidics

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1 Introduction

Microfluidic particle and cell sorting plays an important role in environmental monitoring (Liu et al. 2004; Beyor et al. 2008; Dharmasiri et al. 2010), disease diagnostics (Nagrath et al. 2007; Adams et al. 2008; Hoshino et al. 2011), and therapeutics (Toner and Irimia 2005; Yung et al. 2009). Compared to high-specificity and label-based cell sorting techniques such as fluorescence-activated cell sorter (FACS) (Bonner et al. 1972) and magnetic-activated cell sorter (MACS) (Miltenyi et al. 1990), microfluidic sortings are mostly label-free, relying on cells' intrinsic properties such as size, shape, density, deformability, electric and magnetic properties for manipulation specificity (Pamme 2007; Tsutsui and Ho 2009; Gossett et al. 2010; Lenshof and Laurell 2010). When applicable, microfluidic sortings are favored over label-based ones, because they are inexpensive and require minimal user training for operation (Gossett et al. 2010). Among them, those based on channel design including pinched flow fractionation (Yamada et al. 2004) and deterministic lateral displacement (Huang et al. 2004; Davis et al. 2006) combine laminar flows with mechanical structures to direct particles of different sizes into separate streamlines. Continuous inertial separation uses balance between inertial lift force and Dean drag force in curved channels for size-dependent sorting of particles and cells (Di Carlo 2009). On the other hand, external energy inputs such as acoustic, electric and magnetic forces have also been used to manipulate cells in microfluidic systems. Depending on the application, their simpler channel geometry and faster manipulation speed may outweigh the complications of integrating electrodes in their designs. For example, acoustophoresis can separate particles and cells according to their size, density, as well as compressibility (Laurell et al.

2007; Shi et al. 2009; Wang and Zhe 2011). Dielectrophoresis (DEP), arising from interactions of cells' dipoles and their surrounding electric fields, can realize low-cost and integrated devices for cell manipulation (Voldman 2006). Magnetophoresis (MAP) takes advantages of paramagnetic nature of red blood cells and magnetotactic bacteria and applies non-uniform magnetic fields to separate them from non-magnetic objects (Zborowski et al. 2003; Lee et al. 2004). However, most applications of magnetophoresis use functionalized magnetic beads for labeling (Pamme 2006; Liu et al. 2009; Gijs et al. 2010). The label-based methods are manually intensive and time-consuming. The magnetic moments of these beads, even from the same batch, can vary dramatically due to their manufacturing procedure, making scaling of the method difficult (Hafeli et al. 1997; Miller et al. 2001; Rife et al. 2003; Mihajlovic et al. 2007; Shevkopyas et al. 2007).

To address problems with label-based magnetophoresis, a label-free technique that uses reverse magnetophoresis to manipulate and sort cells has been developed recently based on ferrofluid hydrodynamics (ferrohydrodynamics) (Yellen et al. 2005; Kose et al. 2009; Zhu et al. 2010, 2011a; Kose and Koser 2012). Ferrofluids are colloidal suspensions of magnetic nanoparticles, typically magnetite (Fe_3O_4) with approximately 10 nm diameters (Rosensweig 1985). They are covered by either electrostatic or steric surfactants to keep them from agglomeration due to van der Waals force and in suspension within a water or oil medium. Ferrohydrodynamics studies mechanics of ferrofluid motion under external magnetic fields (Rosensweig 1985; Odenbach 2002). Its applications in microfluidics, recently reviewed by Nguyen (Nguyen 2012), include miniaturized polymerase chain reaction (PCR) (Sun et al. 2007, 2008), traveling-wave magnetic field pumping (Mao and Koser 2006; Mao et al. 2011), micro-scale mixing (Mao and Koser 2007), micropump (Hatch et al. 2001; Love et al. 2004), and droplet manipulation (Nguyen et al. 2006; Zhang et al. 2011a, b).

In applications of cell manipulation, the purpose of using ferrofluids is to induce effective magnetic dipole moments within cells. Under non-uniform magnetic fields, cells will experience in the weaker field direction a magnetic buoyancy force, analogous to buoyancy force, as the magnitude of the force is proportional to the volume of the cell (Rosensweig 1985). Many groups have been working on adapting this principle to particles and cells sorting. For example, Whitesides' group separated synthetic particles according to their densities' difference using paramagnetic salt solutions (Winkleman et al. 2007; Mirica et al. 2009). Pamme's group demonstrated continuous particle and cell manipulation using paramagnetic salt solution in microfluidic devices (Peyman et al. 2009; Rodriguez-Villarreal et al. 2011). Xuan's group studied the transport of particles in both

paramagnetic solutions and ferrofluids through a rectangular microchannel embedded with permanent magnets (Liang et al. 2011; Zhu et al. 2012). Park's group recently sorted human histolytic lymphoma monocytes cells from red blood cells using gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) solution (Shen et al. 2012). However, magnetic susceptibility of paramagnetic salt solutions is inherently small, about five orders of magnitude weaker than that of a ferrofluid (Krebs Melissa et al. 2009), rendering slower manipulation speed and low throughput. As a result of the higher susceptibility of ferrofluids, Koser's group was able to use an integrated microfluidic platform for sorting of microparticles and live cells within a citrate stabilized cobalt-ferrite ferrofluid in static flow conditions (Kose et al. 2009). The same device was also applied to continuous-flow frequency-adjustable particles separation (Kose and Koser 2012). Our group developed high-efficiency and high-throughput continuous-flow particle separation and focusing devices using commercial ferrofluid sand hand-held permanent magnets (Zhu et al. 2010, 2011a, b). Permanent magnet based devices are low-cost and easy to operate; their operations do not generate heat. Magnetic fields produced by permanent magnets are substantially larger than the ones by current-carrying electrodes.

High throughput, label-free and selective cell sorting realized in a single automated device can have profound impacts on environmental monitoring, diagnostics and therapeutics. Although continuous-flow ferrohydrodynamic sorting has been demonstrated with microparticles, it has not previously been reported with live cells (Zhu et al. 2010). The potential for live cell applications of continuous-flow ferrohydrodynamic sorting motivates the study presented here. We developed a microfluidic device that could continuously sort cells of different sizes based on ferrohydrodynamics, which involved manipulation of cells within ferrofluids via external non-uniform magnetic fields. When cell mixtures and ferrofluids were injected into the channel by a pressure-driven flow, deflections of cells from their laminar flow paths would occur because of the magnetic field gradient and resulting magnetic buoyancy force. This deflection will lead to spatial separation of cells of different sizes at the end of channel.

In the following sections, we first summarize materials and methods used in this study, followed by results from a three-dimensional theoretical study of cells' transport in the microfluidic device. Cell viabilities of *Escherichia coli* and *Saccharomyces cerevisiae* in a commercial ferrofluid are then discussed. Afterwards, calibration of the sorting device with fluorescent polystyrene microparticles is performed. *E. coli* and *S. cerevisiae* are sorted in the device, and cells distribution is analyzed on samples collected from channel outlets. In the end we will discuss outlook of ferrohydrodynamic sorting.

2 Materials and methods

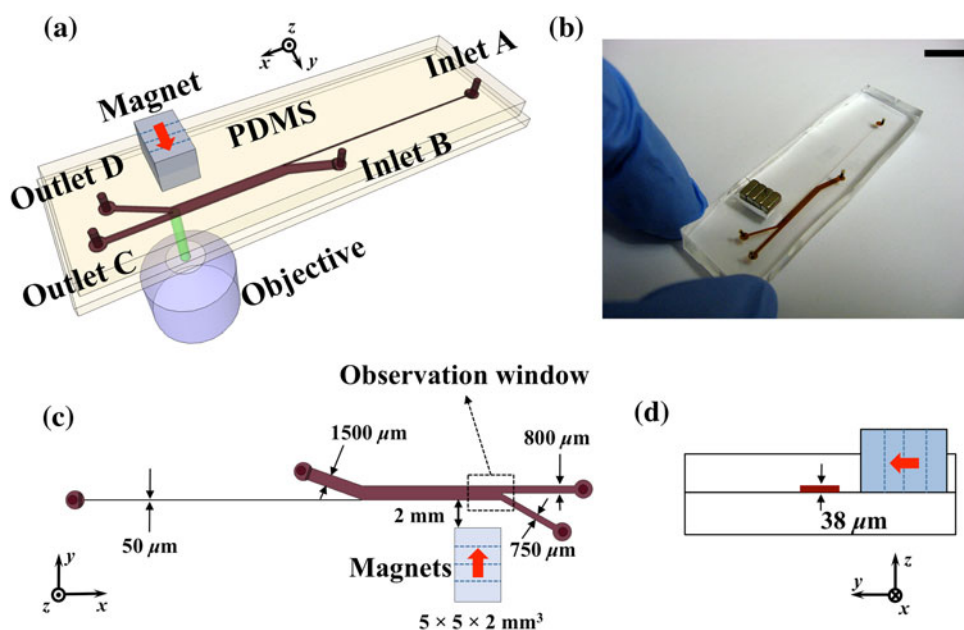
The prototype polydimethylsiloxane (PDMS) microfluidic device was fabricated through a standard soft-lithography approach and attached to a flat surface of another piece of PDMS, as shown in Fig. 1a and b. A mask of the device pattern was created using AutoCAD 2008 (Autodesk Inc., San Rafael, CA, USA) and printed by a commercial photo-plotting company (CAD/Art Services Inc, Bandon, OR, USA). Dimensions of the microfluidic channel are listed in Fig. 1c and d. Thickness of the device was measured to be 38 μm by a profilometer (Dektak 150, Veeco Instruments Inc., Chadds Ford, PA, USA). Before attachment, PDMS surfaces were treated with plasma (PDC-32G plasma cleaner, Harrick Plasma, Ithaca, NY, USA) at 11.2 Pa O_2 partial pressure with 18 W power for 1 min. A stack of four NdFeB permanent magnets was embedded into PDMS channel with their magnetization direction vertical to the channel during curing stage. Each magnet is 5 mm in width, 5 mm in length and 2 mm in thickness. The magnet stack was placed 2 mm away from the channel. Flux density at the center of magnets stack’s surface was measured to be 470 mT by a Gauss meter (Model 5080, Sypris, Orlando, FL, USA) and an axial probe with 0.381 mm diameter of circular active area. Before liquid injection, the device was treated with plasma for 10 min to render PDMS surfaces hydrophilic. This step ensured both cells and microparticles would not attach onto PDMS surfaces during sorting.

We used a commercial water-based, pH ~ 7 magnetite ferrofluid coated with anionic surfactants (EMG 408, Ferrotec Co., NH, USA). Volume fraction of magnetite

particles in this ferrofluid is 1.1 %. Mean diameter of nanoparticles has been determined from transmission electron microscopy (TEM) images to be ~ 10 nm. Initial magnetic susceptibility was measured to be 0.26; saturation magnetization was 6 mT; viscosity was 1.2×10^{-3} kg/m s. We chose to use EMG 408 ferrofluid in live cells sorting, because of its balance between good biocompatibility, reasonable transparency under both fluorescent and bright-field modes microscopy, and moderate magnetic properties. *E. coli* (strain MG1655) and *S. cerevisiae* (Baker’s yeast), and two fluorescent microparticles (green 1.0 μm diameter, Thermo Fisher Scientific Inc., Waltham, MA, and red 7.3 μm diameter, Bangs Laboratories Inc., Fishers, IN, USA) were used in sorting. Ferrofluid and particles/cells mixture injected into microchannel were maintained at tunable flow rates using a syringe pump (Nexus 3000, Chemyx Inc., Stafford, TX, USA). Sorting was conducted on the stage of an inverted microscope (Zeiss Axio Observer, Carl Zeiss Inc., Germany). Micrographs of cells and particles were recorded through either a green fluorescent filter set (41001 FITC, Chroma Technology Corp., Rockingham, VT, USA), or a red filter set (43HE, Carl Zeiss Inc., Germany), and a CCD camera (SPOT RT3, Diagnostic Instruments, Inc., Sterling Heights, MI, USA). Cell samples collected from channel outlets were pipetted onto microscope slides and analyzed using a high-resolution CCD camera (AxioCam HR, Carl Zeiss Inc., Germany) for size distributions to quantitatively evaluate efficiency of this approach. ImageJ[®] software was used to count the number of cells.

Saccharomyces cerevisiae (Baker’s yeast) cells were first grown in a 10 ml test tube containing 2 ml of YPG

Fig. 1 **a** Schematic representation of the sorting device with permanent magnets and a microfluidic channel. **b** An image of prototype device. Scale bar 10 mm. **c** Top view of the device and relevant dimensions. Red arrows indicate direction of magnets’ magnetization. **d** Cross-section of the device



medium (10 g/l yeast extract, 20 g/l glucose, 20 g/l glucose) overnight. They were then transferred into a 100 ml shake flask containing 20 ml of YPG medium. After 4 h growth at 30 °C and 250 rpm, cells in the flask were stained with fluorophores. *E. coli* (strain MG1655) cells were first grown in a 10 ml test tube containing 2 ml of Luria–Bertani (LB) medium overnight. They were then transferred into a 100 ml shake flask containing 20 ml of LB medium (25 g/l LB). After 4 h growth at 37 °C and 250 rpm, cells were stained with fluorophores. Nucleic acid stains SYTO9 (green) and SYTO17 (red) (Molecular Probes Inc., Eugene, OR, USA) were used in cell staining.

To study of viability of *E. coli* and *S. cerevisiae* cells exposed to EMG 408 ferrofluids, nominally 2×10^9 cells *E. coli* and 2×10^7 cells *S. cerevisiae* grown as described above were centrifuged twice at 4 °C and washed in defined M9 medium (6.78 g/l Na_2HPO_4 , 3.0 g/l KH_2PO_4 , 0.5 g/l NaCl, 1.0 g/l NH_4Cl) without carbon source. For either cell type in duplicate, the washed cell pellet from centrifugation was combined with either 2 ml of EMG 408 ferrofluid or 2 ml M9 medium as a control. After 2 h of incubation at room temperature in these fluids, cell density was determined in triplicate using standard microbial serial dilutions (10^6 dilution for *E. coli*, and 10^4 dilution for *S. cerevisiae*), with the transferring of known volumes to Petri plates and counting of Colony Forming Units (CFU) after 24 h.

3 Theory and simulation

Previously, we reported both two-dimensional (2D) and three-dimensional (3D) analytical models for microfluidic transports of microparticles in ferrofluids (Zhu et al. 2011a, b). In this work, we applied the 3D analytical model to predict cells' sorting in permanent magnet based device. Briefly, we obtained cells' trajectories by first calculating magnetic buoyancy force on cells using a 3D analytical model of magnetic fields (Furlani and Sahoo 2006) and a nonlinear magnetization model of ferrofluids (Rosensweig 1985), and then solving governing equations of motion for cells in laminar flow condition (Brody et al. 1996). All relevant parameters used in our simulation are listed in Fig. 1 and Sect. 2. In addition, we calculated volume of a single rod-shape *E. coli* cell with short axis of 0.5–1 μm and long axis of 2–4 μm to be 2.1–16.7 μm^3 (Kaya and Koser 2009), and volume of a single sphere-shape *S. cerevisiae* cell with diameter of 7–9 μm to be 180–382 μm^3 (Jorgensen et al. 2002).

Figure 2 summarizes simulated distribution of magnetic fields and magnetic buoyancy forces in the sorting channel, as well as 3D trajectories of *E. coli* and *S. cerevisiae* cells. The surface plot in Fig. 2a shows magnitude of magnetic

fields of x – y plane at $z = 0$. Magnetic fields decayed rather quickly from the surface of the magnet and formed a gradient that resulted in magnetic buoyancy force on cells in both x and y directions, as indicated in Fig. 2b. Consequently, cells experiencing such force when entering the sorting channel would decelerate in x direction and accelerate in y direction. Force computed on a spherical microparticle of 7.3 μm diameter, with its total volume ($\sim 200 \mu\text{m}^3$) close to that of a single *S. cerevisiae* cell, is on the order of 10 pN. For comparison, buoyant force on the same particle is calculated to be 0.04 pN, considering the difference between particle and ferrofluid densities (particle 1,050 kg/m^3 , ferrofluid: 1,070 kg/m^3). Its magnitude is about 1,000 times smaller than magnetic buoyancy force. Cell mixtures were quickly sorted by magnetic buoyancy force towards the end of channel, as shown in Fig. 2c with simulated cells' trajectories considering their natural size variations. All *E. coli* cells, having much smaller size and volume compared to *S. cerevisiae* cells, exited the channel through Outlet D, while all *S. cerevisiae* cells migrated towards Outlet C. Figure 2d–f illustrate distribution of magnetic fields and forces, as well as trajectories of cells of y – z plane at $x = 0$; Fig. 2g–i depict the cases of x – z plane at $y = 0$. We are interested in 3D trajectories of cells, in part due to the opaqueness of ferrofluids and difficulty in recording cells' weak fluorescence in the channel, especially the red fluorescent from *S. cerevisiae* cells, as shown later in the results. In a concentrated ferrofluid ($\sim 10\%$ v/v), particles and cells are visible only when they are very close ($\sim 1 \mu\text{m}$) to the surface of channel (Zhu et al. 2011b). Visibility was a less of a problem when diluted ferrofluids ($\sim 1\%$ v/v) and thin microchannel were used in our device. Simulation results from Fig. 2f and i indicated in our current setup all cells were pushed towards the channel bottom surface, which would enhance visibility of stained cells.

4 Results and discussions

4.1 Cell viability

Figure 3a shows the CFU in both M9 medium and EMG 408 ferrofluids after incubation. Counts of CFU for each case were averaged over three plates and plotted in Fig. 3b. We observed a slight increase in cell density after 2 h of incubation in the ferrofluid compared to the M9 medium control for both cell types, suggesting a possibility that either the EMG 408 ferrofluid acted as a cell protectant or the cells continued to grow in this ferrofluid during incubation. Nonetheless, this ferrofluid was not detrimental to the viability of both cell types after 2 h of exposure, which allowed enough time to carry out the sorting procedure.

Fig. 2 Analytical three-dimensional simulation of magnetic field and force distributions in microfluidic channel, and trajectories of cells. Simulation parameters match exact experimental conditions. **a–c** x – y plane ($z = 0$), **d–f** y – z plane ($x = 0$), **g–i** x – z plane ($y = 0$) of magnetic field strength (*surface plot*) (**a, d, g**), magnetic force (*surface plot* force magnitude) (**b, e, h**), and particles’ trajectories (**c, f, i**). *Dots* indicate starting points, while *crosses* indicate ending points of cells’ trajectories. *E. coli* cell has volume range of 2.1 – $16.7 \mu\text{m}^3$ and Yeast cell has volume range of 180 – $382 \mu\text{m}^3$, resulting in a distribution of trajectories for each type of cell. *Blue triangle* in **c** indicates boundary between Outlets C and D. *Dots* indicate starting points, while *crosses* indicate ending points of cells’ trajectories

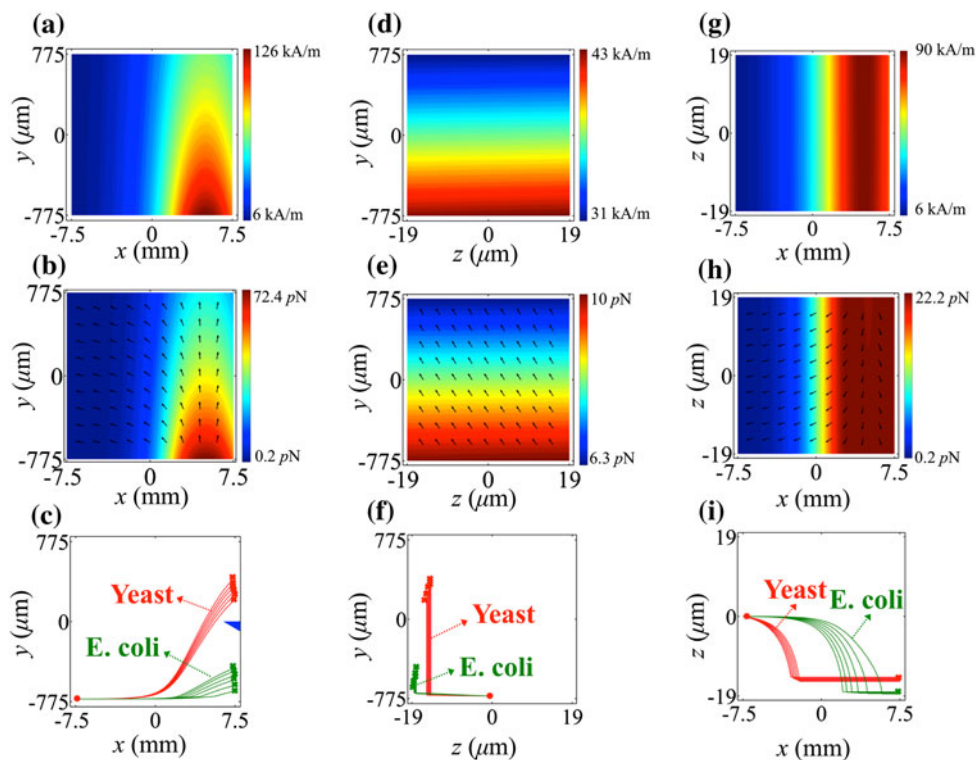
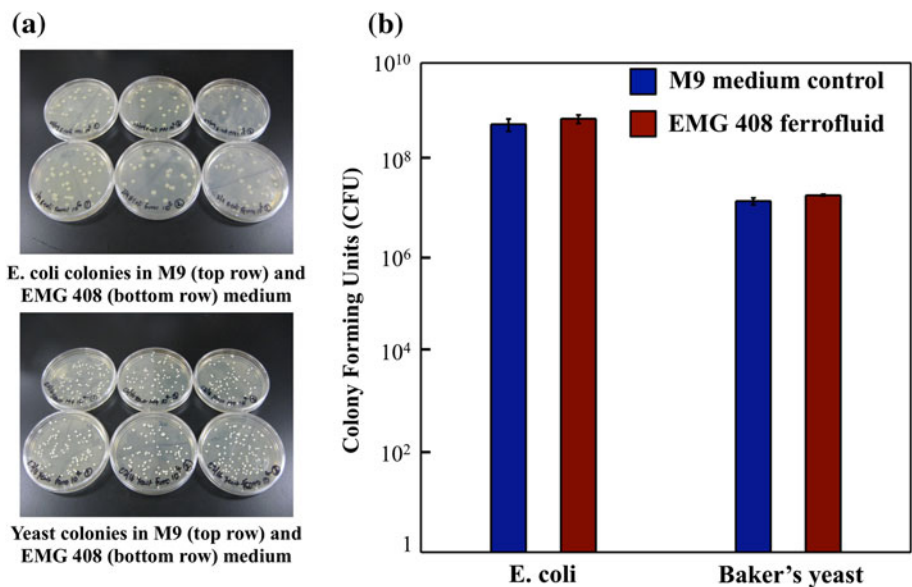


Fig. 3 Cell viability test of *E. coli* and *S. cerevisiae*. **a** Top and bottom photos show *E. coli* and yeast colonies formed in M9 medium and EMG 408 ferrofluids after 10^6 dilution from initial growth, respectively. **b** Colony Forming Units (CFU) count of *E. coli* and *S. cerevisiae* using initial growth cell concentration



4.2 Cells sorting

We first calibrated the sorting device using a mixture of *E. coli* cells and red fluorescent $7.3 \mu\text{m}$ particles, which have similar total volume of *S. cerevisiae* cells. Washed *E. coli* cell pellet from centrifugation as described above was stained with $1 \mu\text{l}$ of green nucleic acid stain SYTO9. Both particles and cells have concentrations of $\sim 10^7$ counts/ml. We introduced microparticles/cells mixture into

microfluidic channel Inlet A at a constant flow rate of $1.5 \mu\text{l}/\text{min}$. The mixture was hydrodynamically focused into a narrow stream by sheath flow from Inlet B at a flow rate of $6 \mu\text{l}/\text{min}$. The observation window was located right before the channel outlets, as indicated in Fig. 1c. When magnetic fields were not present, particles and cells were observed in fluorescent mode flowing together near side-wall of the channel and exiting through Outlet D, as shown in composite micrograph of Fig. 4a. When magnetic fields

were present, magnetic buoyancy forces deflected particles from their laminar flow paths towards Outlet C, as shown in Fig. 2b. On the other hand, forces on smaller *E. coli* cells were inadequate to deflect them to Outlet C; therefore they exited the channel through Outlet D still, as shown in Fig. 2c. This resulted in spatial separation of particles/cells mixture at the end of channel. We were able to separate $\sim 10^6$ particles from $\sim 10^6$ cells/h with 1.5 $\mu\text{l}/\text{min}$ flow rate. Simply increasing the flow rate can further increase sorting throughput. Current microfluidic sorting schemes use flow rates ranging between ~ 10 $\mu\text{l}/\text{min}$ and ~ 1 ml/min (Gossett et al. 2010). With such flow rates and 10^7 – 10^8 cells/ml concentration, maximum sorting throughput of our device in theory can go up to 10^9 cells/h. Increasing the flow rate will increase overall cell sorting throughput, at the same time it will decrease cell sorting efficiency if same device geometry is used. It is because now cells of large sizes do not have enough time in the channel to be fully deflected and separated from cells of smaller sizes. A possible way to increase both cell sorting throughput and efficiency is to apply high flow rates and use longer channels.

Secondly, we calibrated the device using a mixture of *S. cerevisiae* cells and green fluorescent 1.0 μm particles, which have similar volume as *E. coli* cells. *S. cerevisiae* were stained with red nucleic acid stain SYTO17. Both particles and cells again have concentrations of $\sim 10^7$ counts/ml. Due to weak red fluorescence from SYTO17 in our setup, we chose to use a combination of bright-field and fluorescent modes microscopy to record the sorting process. Figure 4d shows merged composite micrograph of green fluorescent 1.0 μm particles and bright-field particles/*S. cerevisiae* mixture, both of which exited channel through Outlet D when magnetic fields were

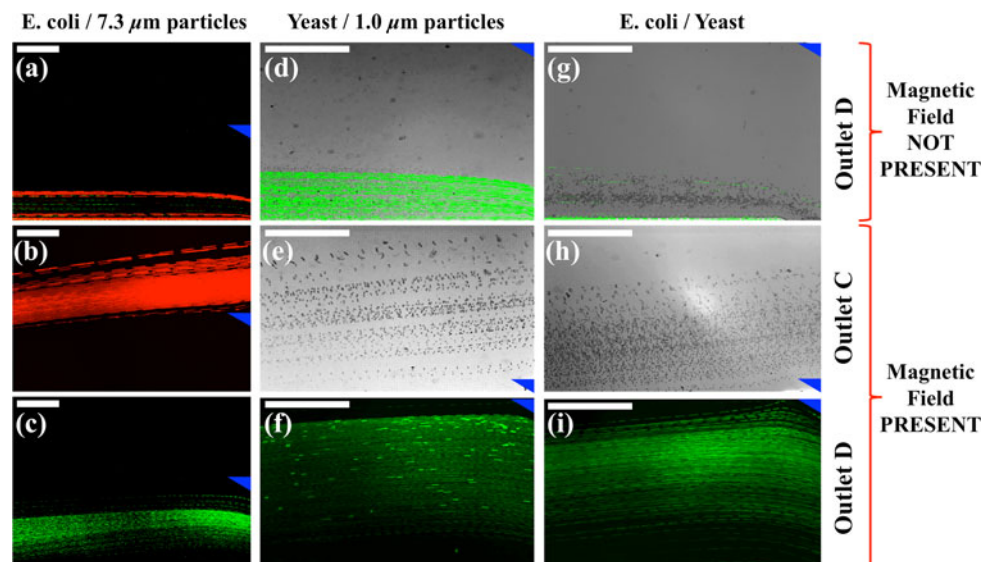
off. Sorting of this mixture was achieved as soon as magnetic fields were on, as depicted in Fig. 4e and f. Cells distribution analysis presented in the following section confirmed a close to 100 % sorting efficiency. Sorting throughput was $\sim 10^6$ cells/h. Here we demonstrated that combination of bright-field and fluorescent microscopy can successfully circumvent recording issues originating from opaqueness of ferrofluids and weak fluorescence from stained live cells.

Finally, sorting of *E. coli* and *S. cerevisiae* cells were carried out in the same device at the same time. *E. coli* cells were stained with green fluorescence while *S. cerevisiae* were stained with red fluorescence. Both types of cells were adjusted to $\sim 10^7$ cells/ml concentration in initial mixture. It is clearly shown in Fig. 4g that all cells exited from the channel through Outlet D when there was no magnetic field. Both bright-field and fluorescent mode micrographs of cells were recorded and merged to form Fig. 4g. *S. cerevisiae* cells were successfully sorted from the initial cell mixture with the application of magnetic fields, as demonstrated in Fig. 4h and i.

4.3 Cell sorting efficiency

In order to precisely evaluate sorting efficiency, we collected samples from both Outlets C and D and analyzed them for size distributions off chip. We stained cells in distinctive fluorescence and counted them using ImageJ[®] software. Specifically, in first calibration, *E. coli* cells were green and 7.3 μm particles were red; in second calibration, *S. cerevisiae* cells were red and 1.0 μm particles were green; in cells sorting, *S. cerevisiae* cells were red and *E. coli* cells were green. Fluorescent mode was chosen for distribution analysis to avoid miscounting of cell types in

Fig. 4 Experimental composite micrographs of sorting process. **a, d, g** were particles/cells mixture **a** *E. coli* (green) and 7.3 μm particles (red); **d** *S. cerevisiae* (red and bright-field) and 1.0 μm particles (green); **g** *E. coli* (green) and *S. cerevisiae* (red and bright-field) before magnetic fields were applied. **b, e, h** were micrographs of Outlet C after magnetic fields were applied, and **c, f, i** were micrographs of Outlet D. Blue triangles indicate boundary between Outlets C and D. Scale bars represent 200 μm



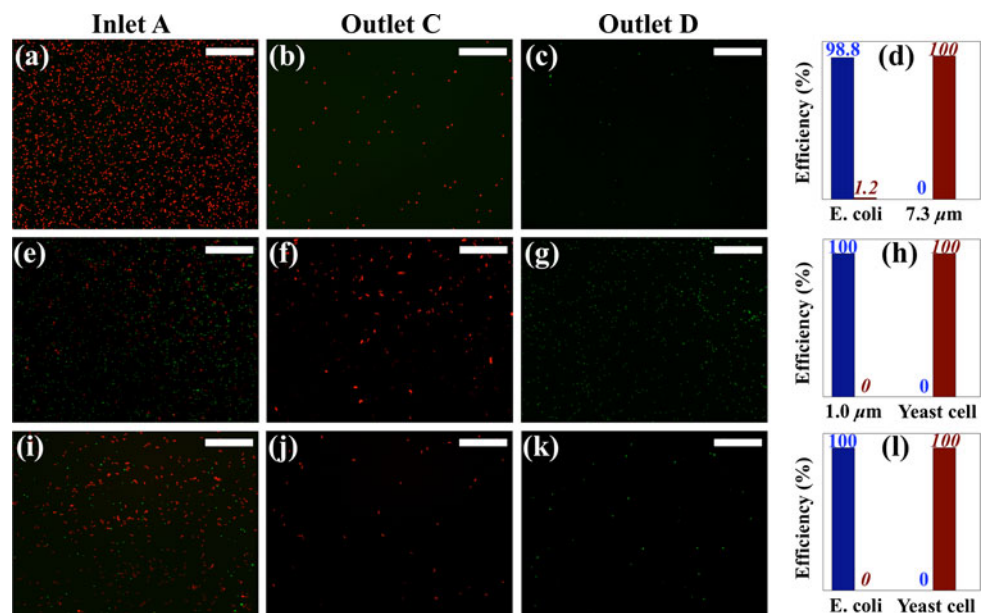
bright-field micrographs. A magnetic field was applied to push all particles and cells onto a surface of glass slide to increase visibility. We define remaining efficiency as ratio of number of particles or cells exiting from Outlet D after magnetic field application to their initial number before magnetic field application. Similarly, sorting efficiency is defined as the ratio of number of particles or cells exiting from Outlet C after magnetic field application to their initial number before magnetic field application. Figure 5a shows a representative composite micrograph of *E. coli* cells and 7.3 μm particles collected from Inlet A before sorting. 100 % of 7.3 μm particles migrated to Outlet C and 98.8 % *E. coli* cells remained in Outlet D, as depicted in Fig. 5b and c. Remaining and separation efficiencies for both particles are plotted in Fig. 5d. Figure 3e–l show micrographs and efficiencies for *S. cerevisiae* cells/1.0 μm particles mixture sorting and *S. cerevisiae* cells/*E. coli* cells mixture sorting, respectively. Both cases have 100 % efficiencies. It should be noted that samples collected from Outlets C and D were greatly diluted by ferrofluid sheath flow from Inlet B, rendering much lower particles and cells concentration for distribution analysis. A possible solution to this problem is integration of cell focusing (Zhu et al. 2011a) and sorting steps on one chip. The resolution of ferrohydrodynamic sorting depends on the difference in the cells' deflections towards the outlets. There is more deflection if cells stay longer in the channel. Cells stream typically has a finite width in the channel due to device design and their diffusions. In order to separate two types of cells, the difference in the cells' deflections needs to be larger than the width of cells stream at outlets. In the case of small size difference between two types of cells, a longer channel may help enhance sorting efficiency.

Ferrohydrodynamic cell sorting throughput and efficiency greatly depend on device parameters and fluid properties. Device parameters including dimensions of magnets and channel, flux density of magnets, relative positions of magnets with respect to channel, as well as fluid properties including ferrofluid concentration, viscosity, and susceptibility have significant effects on sorting performance. Optimizations of these parameters are needed in order to extend this approach into manipulations and sorting of different types of cells. For this purpose, we are currently studying a comprehensive three-dimensional model that numerically simulates effects of all these parameters on cell sorting performance. Results from this study will be summarized and published elsewhere.

4.4 Outlooks of ferrohydrodynamic sorting

Ferrohydrodynamic cell sorting offers the potential for high throughput (~10⁶ cells/h in this study and ~10⁹ cells/h in theory) and high separation efficiency (~100 %) that are comparable to existing microfluidic sorting techniques but without the use of labels. The associated device is inexpensive and simple, only requiring a channel and hand-held permanent magnets. Sorting specificity of this approach is not limited to size difference only; it is also sensitive to cells' shape and deformability (Kose et al. 2009). In adapting it to miniaturized flow cytometry, ferrohydrodynamic manipulation can first focus cells into single cell streams before sorting, eliminating needs for excessive sheath flow and preventing sample dilution (Zhu et al. 2011a). Compared to paramagnetic solution based sorting, ferrofluid offers much higher magnetic susceptibility, eliminating needs for either

Fig. 5 Experimental composite micrographs of size distribution analysis, including micrographs of particles/cells mixture collected before sorting at Inlet A and after separation at Outlets C and D, and remaining and separation efficiencies. **a–d** were for *E. coli* and 7.3 μm particles mixture; **e–h** were for *S. cerevisiae* and 1.0 μm particles mixture; **i–l** were for *E. coli* and *S. cerevisiae* mixture. Blue bar with normal number on top shows remaining efficiency, while red bar with italic number on top shows separation efficiency. Scale bars represent 200 μm



microfabricated ferromagnetic structures to enhance field gradient or hypertonic concentrations of paramagnetic salts that are not biocompatible for live cell manipulation.

On the other hand, using water-based ferrofluids for cell manipulation is a work in progress. Diagnostic and research applications directed towards simply purifying or isolating cells of interest from complex mixtures such as blood and exfoliated cytology specimens are exciting. For instance, blood cells obscure the detection of the larger but rare abnormal cervical cells from Pap test specimens and metastatic epithelial tumor cells circulating in blood (Moriarty et al. 2009; Yu et al. 2011). Misinterpreted cervical cytology ranks third among causes of medical negligence claims against pathologist (Frable 2007). A simple, low-cost tumor cell enrichment platform would benefit cancer screening. However, two issues, cell visibility and biocompatibility of mammalian cells in ferrofluids, limit applications of ferrohydrodynamic manipulation. Ferrofluids are opaque due to light diffraction from their high concentration of magnetic nanoparticles. Fluorescent cells need to be close to channel surface for microscopic recording. In order to address this issue, ferrofluids with low solid content, as well as shallow microfluidic channel, are favored for cell manipulation. In addition, magnetic fields can be used to push cells onto channel surface, increasing visibility of cells in fluorescent mode. In this study, we used a combination of both bright-field and fluorescent modes microscopy to circumvent the opaqueness issue. Cells were readily visible in a shallow channel in bright-field micrographs. Another potential issue is biocompatibility of ferrofluids. Our next step is to extend this methodology to mammalian cells, particularly human specimens such as blood and other bodily fluids, exfoliated musical cells, and tumor aspirates. The requirements of mammalian cells may differ from *E. coli* and *S. cerevisiae*. For cell manipulation, materials, pH value, and surfactants of ferrofluids need to be rendered biocompatible, at the same time the overall colloidal system of ferrofluids must be maintained. Typically, nanoparticles within ferrofluids for cell applications are made of magnetite (Pankhurst et al. 2003). pH value of ferrofluids needs to be compatible with cell culture and maintained at 7.4. Salt concentration, tonicity, and surfactant must be carefully chosen close to physiological conditions to reduce cell death. Although these are stringent requirements, progress has been made towards synthesizing biocompatible ferrofluids. For example, Koser's group used citrate to stabilize cobalt-ferrite nanoparticles for live red blood cell and *E. coli* cell sorting (Kose et al. 2009). Yellen's group used bovine serum albumin (BSA) to stabilize magnetite nanoparticles for human umbilical vein endothelial cells manipulation (Krebs Melissa et al. 2009). Viability tests from both studies have shown cells were

able to retain their viability for up to several hours in ferrofluids. In our study, a commercially available pH ~ 7 magnetite ferrofluid was able to sustain viability of both *E. coli* and *S. cerevisiae* cells for at least 2 h.

5 Conclusion

In conclusion, we have developed a label-free and continuous-flow ferrohydrodynamic cell sorting device and applied it in separating *E. coli* and *S. cerevisiae* cells. A commercial magnetite ferrofluid was used to separate particle and cell mixtures. Construction of our device is simple and low-cost; we choose to use permanent magnets instead of integrated electrodes to eliminate complex microfabrication process and auxiliary power supply. Current sorting throughput is 10^7 cells/h, and sorting efficiency is close to 100 %. We envision this device can achieve up to two orders higher throughput while still maintaining current sorting efficiency. This device can also be used for mammalian cells sorting and enrichment with a biocompatible ferrofluid.

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