Dielectrophoretic manipulation of drops for high-speed microfluidic sorting devices

Keunho Ahn and Charles Kerbage
DEAS, Harvard University, Cambridge, Massachusetts 02138

Tom P. Hunt
Department of Physics, Harvard University, Cambridge, Massachusetts 02138

R. M. Westervelt
DEAS and Department of Physics, Harvard University, Cambridge, Massachusetts 02138

Darren R. Link
DEAS and Department of Physics, Harvard University, Cambridge, Massachusetts 02138 and RainDance Technologies, Guilford, Connecticut 06437

D. A. Weitz[a)]
DEAS and Department of Physics, Harvard University, Cambridge, Massachusetts 02138

(Received 29 August 2005; accepted 15 December 2005; published online 12 January 2006)

We demonstrate a high-throughput drop sorter for microfluidic devices that uses dielectrophoretic forces. Microelectrodes underneath a polydimethylsiloxane channel produce forces of more than 10 nN on a water drop in an inert oil, resulting in sorting rates greater than 1.6 kHz. We investigate the dependence of such forces on drop size and flow. Alternate designs with electrodes on either side of a symmetric channel *Y* junction provide refined control over droplet selection. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2164911]

Water drops dispersed in an inert, immiscible solvent are very promising for use as well-defined, confined microreactors.[1] In one particularly promising example, water drops in oil, or emulsions, have been used to carry out *in vitro* transcription and translation of single genes to create new enzymes.[2] Microreactors can be the basis of a very efficient means of directed evolution: sorting genes under environmental pressure for improved or modified functionality and catalytic activity of new enzymes. Such directed evolution requires accurate control of femtoliter volumes, easily achieved by using micron-sized emulsion drops, to increase the effective concentration of the single gene in each drop and to enable high-throughput screening of huge libraries of genes. While bulk emulsions provide the requisite encapsulation, effective high-throughput screening requires a much higher degree of control, with access to individual microreactors. Such control of microreactors is best achieved using microfluidic technology,[1] which enables formation of uniform drops,[3] passive drop manipulation,[4] complex emulsification,[5] and mixing of small volumes.[1] However, high-speed sorting of drops in the microfluidic device is essential. For example, sorting a typical library of 10^8–10^9 genes demands throughput of at least 1 kHz to be practical. Microfluidic sorters have been made for particles and cells in continuous water flow using electro-osmotic,[6] mechanical,[7] optical,[8] magnetic,[9] electrophoretic,[10] and dielectrophoretic actuation.[11] Microfluidic sorters for drops have been made with mechanical actuation, but this is limited to 10 Hz Ref. 12; they have also been achieved using charge, but this requires an electrochemical reaction within the drops.[10,13] Electro-osmotic forces are not appropriate for drop sorting because oil is nonconducting. Magnetic sorting can achieve high rates but requires magnetic labeling. Thus, improved drop sorting speed in microfluidic systems is essential for development of high-throughput microreactors.

In this letter, we report high-speed sorting of water drops in microfluidic devices using dielectrophoresis. We characterize the dielectrophoretic force by measuring the dependence of the drop velocity on the drop size and the applied voltage. To further improve the sorting, we use a three-electrode device that can pull drops to either side of a symmetric junction.

We form water drops in oil by hydrodynamic flow focusing,[3] where two streams of oil and one of water are focused at the input of the device, as shown in Fig. 1(a). The water drops flow downstream to a *Y* junction. With no electric field, all drops flow into the waste channel which is shorter, and thus offers lower hydrodynamic resistance than the second, collect channel. To direct drops into the collect stream, we energize electrodes under the channel in the sort-

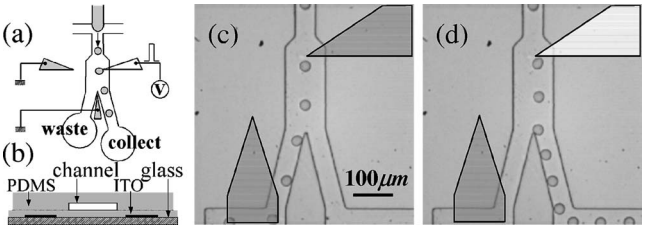

FIG. 1. (a) Schematic top view of the device. Water drops formed by flow focusing in the continuous phase of oil flow into the waste channel since the resistance of the waste channel is smaller than that of the collect channel. (b) Schematic cross section of the device. The molded PDMS microfluidic channel is aligned to the 30-μm PDMS layer which is spin coated on the patterned ITO electrodes. (c) In the absence of an electric field, water drops flow into the waste channel. (d) Applying an electric field, the drops are attracted toward the energized electrode and flow into the collect channel. Transparent ITO electrodes have been drawn in gray for grounded electrodes and white for energized electrodes.

[a)]Electronic mail: weitz@deas.harvard.edu

ing region; the dielectrophoretic force pulls the drops into the collect stream. The electrodes are located with their tips or edges close to the center of the channel to maximize the force exerted on the drops. We use a finite-element simulation (Maxwell 3-D, Ansoft) to estimate the dielectrophoretic forces on water drops for the actual device geometry; this shows that sharp edges of electrodes generate the largest field gradients and forces on the drops. Passing through the *Y* junction, drops can deform and break, with each daughter drop flowing into a separate exit channel. Drops will break due to extensional flow if the capillary number, which characterizes the ratio of viscous to capillary forces, exceeds a critical value.[4] To avoid drop breakup, we design the channel width near the *Y* junction to be bigger than the diameter of the undeformed drop, preventing breakup by decreasing both velocity and extension.

The microfluidic device is fabricated using standard soft lithography methods.[14] A channel pattern of 25 μm thick negative photoresist is produced by UV photolithography on a silicon wafer. A mixture of polydimethylsiloxane (PDMS) elastomer and crosslinker with a weight ratio of 5:1 is molded onto the channels and peeled off after being partially cured. Another mixture with a weight ratio of 20:1 is spincast at 3000 rpm to a 30-μm film on a glass substrate, on which has been patterned indium tin oxide (ITO) electrodes, and also partially cured. The PDMS mold is bonded to the PDMS-coated ITO-glass substrate and fully cured to enhance bonding between the two layers.[15] A schematic cross section of the sorting region of the fabricated microfluidic device is shown in Fig. 1(b).

We use this device to produce drops of water in hexadecane (dielectric constant, $\epsilon_{\text{oil}}=1.8\times10^{-11}$ Farad/m; viscosity, $\eta_{\text{oil}}=8.0\times10^{-3}$ Pa s). We add 5 wt % surfactant (SPAN80) to prevent coalescence. The size of the water drops is controlled by adjusting flow rates of oil and water using syringe pumps (Harvard Apparatus).[3] We form water drops with radii from 2 to 30 μm using water flow rates from 1 to 6 μl/hr and oil flow rates from 100 to 1000 μl/hr. We apply ac voltage up to 2 kV across the electrodes; this avoids screening effects, which are found to weaken a dc field. Drop movement is recorded by a high-speed camera at a frame rate of 20 kHz.

In the absence of electric field, the water drops flow along the path of lower hydrodynamic resistance to the waste channel as shown in Fig. 1(c). When we turn on the voltage, the electric field elongates and displaces the drops toward the electrode located close to the drop stream [Fig. 1(d)]. To quantify the forces on the drops, we measure the average velocity of drops pulled in the direction perpendicular to their flow while varying the applied voltage and the drop size. The average velocity is obtained by measuring the displacement of the drops as a function of time from each frame of the movies recorded by the high-speed camera. The average velocity is proportional to the square of the applied voltage and the drop size (Fig. 2). The velocity is determined by the dielectrophoretic force acting on a drop,[16] $\vec{F}=\vec{m}\cdot\nabla\vec{E}$, where $\vec{m}$ is the dipole moment of the particle and $\vec{E}$ is the electric field. For a spherical particle of radius r, in an oil of dielectric permittivity ϵ_{oil}, the induced dipole moment is given by $\vec{m}=4\pi\epsilon_{\text{oil}}\,\text{Re}[CM(\omega)]r^3\vec{E}$, where $\text{Re}[CM(\omega)]$ is the Claussius–Mossotti factor. For water drops in oil and for frequencies from dc to several MHz, $\text{Re}[CM(\omega)]=1$; this is the case for our experiments, where we apply frequencies of several kHz. The dielectrophoretic force on the drops is balanced by the viscous or Stokes drag, $\vec{F}_S=6\pi\eta_{\text{oil}}r\vec{v}$. Thus, the velocity of the drop is $\vec{v}=\epsilon_{\text{oil}}r^2\nabla\vec{E}^2/3\eta_{\text{oil}}$. This gives $\vec{v}=\epsilon_{\text{oil}}r^2kV^2/3\eta_{\text{oil}}$, where V is the applied voltage and k is a geometric factor determined by electrode shape and location. Using the value of k determined from the finite-element simulation, the maximum dielectrophoretic force on a 12-μm-diam drop at 1 kV is estimated to be 10 nN near the electrode edge, resulting in a maximum drop velocity of 1.0 cm/sec. This is in good agreement with the experimental value as shown in Fig. 2.

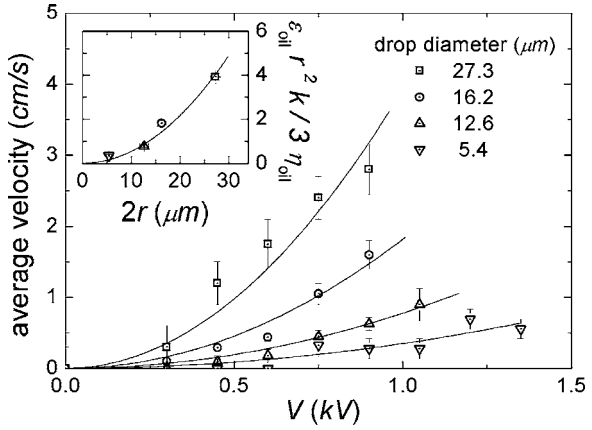

FIG. 2. Average terminal velocity of the water drops as a function of the applied voltage for different drop sizes. The solid lines are best-fit curves with Eq. (4). The inset shows that the parameter, $\epsilon_{\text{oil}}r^2k/3\eta_{\text{oil}}$, obtained from the first fit is proportional to the square of the drop size.

To further refine the description of drop movement, we use a least-squares fit of all the data for the velocity as a function of V, for each drop size, shown as solid lines in Fig. 2. From these fits, we obtain values for $\epsilon_{\text{oil}}r^2k/3\eta_{\text{oil}}$, which are plotted as a function of r in the inset of Fig. 2. They exhibit the expected dependence on r^2 and a least-squares fit gives a value for $\epsilon_{\text{oil}}k/3\eta_{\text{oil}}$, from which we obtain the average geometric factor of the device, $k_{\text{avg}}=2.9\times10^{11}$ m^{-3}. This is in good agreement with values obtained from simulation, which range from 2.0×10^{10} to 2.0×10^{11} m^{-3} near the electrode. The deviation between simulation and experiment likely stems from the highly nonuniform electric field near the electrode edges or from the variation of the PDMS layer thickness at the channel bottom, which determines the distance of the water drops from the electrode and affects the electric-field strength in the channel. The overall good agreement confirms that finite-element techniques can be used to help optimize electrode design for microfluidic devices.

The maximum rate of dielectrophoretic sorting depends on the force generated by the electric-field gradient which determines the terminal speed of the drops. The time for the drops to attain the terminal velocity is given approximately by $t=v/a=v/[F/(\Delta\rho 4\pi/3r^3)]=2\Delta\rho r^2/9\eta$, where $\Delta\rho$ is the density mismatch between the water and the oil. Thus, a 1 nN force acting on a 12-μm-diam drop will accelerate the drop to its terminal velocity within 5 μs, much faster than other time scales in the system; therefore inertial effects do not limit the sorting rate. Similarly, the capacitive time constant (RC) of the circuit is small, on the order of 10 ns ($R\sim200\ \Omega$, $C\sim50$ pF), and also does not limit sorting rates.

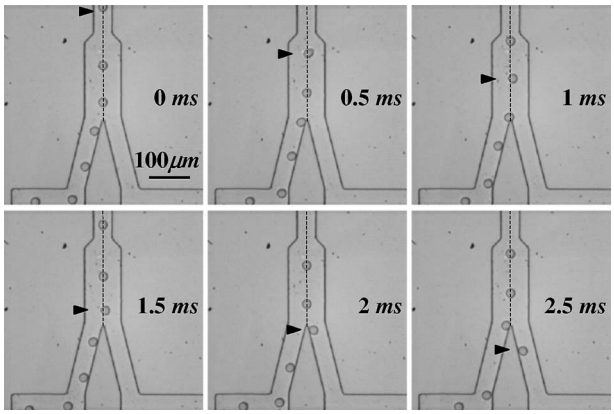

FIG. 3. A single drop sorted out of 1.6 kHz drop flow. Images captured every 500 μs. The arrow marks the drop of interest. One drop directed to the collect channel out of the streamline by a 500-μs pulse of electric field. The transparent electrode location same as Fig. 1.

Instead, for a given velocity, the sorting rate is limited by the on time required for the electric field to displace the drops a distance, d, away from the center of the streamline into the collection channel. This distance is close to zero in the case of symmetric output channels where drop flow direction is evenly distributed into both channels. In the case of asymmetric channels, d is determined by the relative difference of the amount of liquid separated into two output channels, which is proportional to the relative difference of the length of the two output channels. Therefore, $d \sim w\Delta l/l$, where w is width of the channel, l is the total length of the collection and waste channel, and Δl is the difference between the channels. In our device, $d \sim 6$ μm; since the dielectrophoretic force moves 25-μm-diam drops at $v=4$ cm/sec, drops can be sorted in 250 μs. The time scale to displace the drops can be shortened by increasing V or improving the device design. However, an excessively sharp localized field gradient around the electrode ultimately results in drop breakup, setting the limit for the sorting rate in this device. For 25-μm-diam drops, we use square ac pulses with an amplitude of 700 V, a frequency of 10 kHz, and a duration of 500 μs; this enables us to sort a single drop into the collect channel from a continuous stream at rates of 1.6 kHz, as shown in Fig. 3.

Improved sorting speed can be attained by decreasing d. We use a symmetric Y junction with equal fluidic resistance in each output channel, significantly reducing d. However, this device requires more than two electrodes to change the direction of the electric-field gradient with respect to the flow. For example, with three electrodes in a triangular arrangement, each edge can be the highest electric-field region by choosing one of them as a cathode while the other two are grounded. Our device for bidirectional drop manipulation has two output channels with the same length and hydrodynamic resistance, and three electrodes aligned to the middle channel, as shown in Fig. 4. In the absence of electric field, drops are evenly distributed into both channels, as shown in Fig. 4(a). However, upon application of a field to the appropriate electrode, drops can be directed to either one of the two channels, as shown in Figs. 4(b) and 4(c). Optimization of such devices should improve sorting rates.

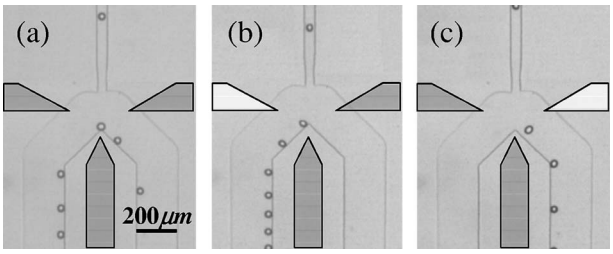

FIG. 4. Bidirectional manipulation of drops. (a) In the absence of the electric field, two outlet channels are symmetric, the junction is an unstable equilibrium, and drops flow to both directions. (b) Drops flow to the left channel with the left electrode energized. (c) Drops flow to the right channel with the right electrode energized. Transparent ITO electrodes have been drawn in gray for grounded electrodes and white for energized electrodes.

Our microfluidic drop sorting devices can be used as an essential component in a platform for high-throughput screening bioassays. Dielectrophoretic forces provide high-speed sorting with no moving parts, and no requirement for droplet charging. We have achieved rates as fast as 4 kHz, but even this is not the ultimate limit. Sorting rates can certainly be increased further by using shorter pulses, higher fields, or improved device design.

This work was supported by the NSF Grant No. (DMR-0243715) and through the Harvard MRSEC Grant No. (DMR-0213805), and by a partial fellowship to K.A. from the Korean Research Foundation. This work was also supported by a gift from Philip Morris and by the Harvard NSEC under NSF Grant No. PHY-0117795.

[1] H. Song, J. D. Tice, and R. F. Ismagilov, Angew. Chem., Int. Ed. **42**, 768 (2003).
[2] D. S. Tawfik and A. D. Griffiths, Nat. Biotechnol. **16**, 652 (1998).
[3] S. L. Anna, N. Bontoux, and H. A. Stone, Appl. Phys. Lett. **82**, 364 (2003).
[4] D. R. Link, S. L. Anna, D. A. Weitz, and H. A. Stone, Phys. Rev. Lett. **92**, 054503 (2004).
[5] A. S. Utada, E. Lorenceau, D. R. Link, P. D. Kaplan, H. A. Stone, and D. A. Weitz, Science **308**, 537 (2005).
[6] P. S. Dittrich and P. Schwille, Anal. Chem. **75**, 5767 (2003).
[7] An Y. Fu, H.-P. Chou, C. Spence, F. H. Arnold, and S. R. Quake, Anal. Chem. **74**, 2451 (2002).
[8] M. M. Wang, E. Tu, D. E. Raymond, J. M. Yang, H. Zhang, N. Hagen, B. Dees, E. M. Mercer, A. H. Forster, I. Kariv, P. J. Marchand, and W. F. Butler, Nat. Biotechnol. **23**, 83 (2005).
[9] H. Lee, Y. Liu, E. Alsberg, D. E. Ingber, R. M. Westervelt, and D. Ham, IEEE International Solid-State Circuits Conference, 2005 (unpublished).
[10] D. R. Link and D. A. Weitz (unpublished).
[11] T. P. Hunt, H. Lee, and R. M. Westervelt, Appl. Phys. Lett. **85**, 6421 (2004).
[12] T. Thorsen (unpublished).
[13] T. Kawano, N. Kanai, S. Ando, M. Yamamoto, J. Fujiwara, T. Torii, and T. Higuchi, Proceedings of the Eighth International Conference on Miniaturized Systems for Chemistry and Life Sciences, Sweden, 2004 (unpublished).
[14] J. C. McDonald, D. C. Duffy, J. R. Anderson, D. T. Chiu, H. K. Wu, O. J. A. Schueller, and G. M. Whitesides, Electrophoresis **21**, 27 (2000).
[15] M. A. Unger, H.-P. Chou, T. Thorsen, A. Scherer, and S. R. Quake, Science **288**, 113 (2000).
[16] H. A. Pohl, *Dielectrophoresis* (Cambridge University Press, Cambridge, 1978).