

## Microreactors

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### Electric Control of Droplets in Microfluidic Devices\*\*

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The precision manipulation of streams of fluids with microfluidic devices is revolutionizing many fluid-based technolo-

gies and enabling the development of high-throughput reactors that use minute quantities of reagents. However, as the scale of these reactors shrinks, contamination effects due to surface adsorption and diffusion limit the smallest quantities that can be used. The confinement of reagents in droplets in an immiscible carrier fluid overcomes these limitations, but demands new fluid-handling technology. We present a platform technology based on charged droplets and electric fields that enables electrically addressable droplet generation, highly efficient droplet coalescence, precision droplet breaking and recharging, and controllable droplet sorting. This is an essential enabling technology for a high-throughput droplet microfluidic reactor.

Networks of small channels are a flexible platform for the precision manipulation of small amounts of fluids.<sup>[1,2]</sup> The utility of such microfluidic devices depends critically on enabling technologies such as the microfluidic peristaltic pump,<sup>[3]</sup> electrokinetic pumping,<sup>[4,5]</sup> and dielectrophoretic-pump or electrowetting-driven<sup>[6]</sup> flow; these technologies can form the essential building blocks for the assembly of fluid-handling modules.<sup>[7]</sup> These modules can be used to perform a variety of key tasks including the measurement of precise aliquots of fluids, the combination of fluid streams, and the mixing of multiple fluid components. The assembly of such modules into complete systems provides a convenient and robust way to construct microfluidic devices. These have myriad uses; for example, high-throughput screening,<sup>[8]</sup> the exploration of chemical phase diagrams, assays of biological molecules,<sup>[9–11]</sup> single-cell analysis,<sup>[12–17]</sup> and combinatorial approaches to protein crystallization<sup>[18]</sup> can all be performed with only minimal consumption of reagents. However, virtually all microfluidic devices are based on flows of streams of fluids; this sets a limit on the smallest volume of reagent that can be used effectively because of the contaminating effects of diffusion and surface adsorption. As the dimensions of small volumes are decreased, diffusion becomes the dominant mechanism for mixing leading to dispersion of reactants. Moreover, surface adsorption of reactants, although small, can be highly detrimental at low concentrations and small volumes. As a result current microfluidic technologies cannot be reliably used for applications involving minute quantities of reagent—for example, bioassays at levels down to the single molecule are not easily performed. An approach that overcomes these limitations is the use of aqueous droplets in an immiscible carrier fluid;<sup>[19]</sup> these droplets provide a well-defined, encapsulated microenvironment that eliminates cross-contamination or changes in concentration caused by diffusion or surface interactions. Droplets provide the ideal microcapsule that can isolate reactive materials, cells, or small particles for further manipulation and study. Moreover, by making droplets as small as one femtoliter, reactions of single biomolecules can be investigated. However, essentially all enabling technology for microfluidic systems developed thus far has focused on single-phase fluid flow, and there are few corresponding, active means to manipulate droplets. In particular, manipulating, mixing, and combining reagents in microfluidic geometries is much more difficult for droplets than for single streams,<sup>[19,20]</sup> especially when the droplets are stabilized with

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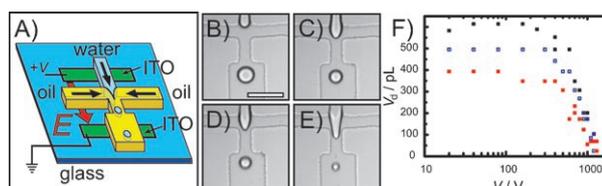
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surfactant, as they must be for more-complex processing. This difficulty has precluded the widespread use of droplet technology as robust microreactors in microfluidic devices.

Herein we present a generic and robust platform technology for manipulating and controlling individual droplets in microfluidic devices. By combining electrostatic charge on the droplets and electric fields on the devices, we develop modules that create, recombine, split, and sort droplets one by one, thus providing fine control over individual microreactors while retaining high purity and enabling very high throughput. By incorporating the forces that result from charging the aqueous fluid in an electric field  $E$ , we can produce smaller droplets with more precise control of their individual timing than is feasible with other strategies that rely solely on viscous forces to overcome surface tension;<sup>[21–25]</sup> this provides a robust droplet-generation module that allows us to produce microreactors with volumes as small as femtoliters, while using orifices whose dimensions are significantly larger than the one-micron-diameter droplet size, thereby reducing potential clogging problems. By incorporating charges of opposite sign on different droplets we can merge droplets controllably and reliably, thus overcoming the stabilizing forces caused by surface tension and lubrication; this provides a robust droplet-coalescence module that allows us to mix precise aliquots of reactants. By incorporating the extensional force induced by an electric field we can controllably split large droplets into smaller aliquots for further analysis and simultaneously recharge neutral drops for further processing; this provides a robust splitting or charging module that allows us to perform multiple assays on the same materials. By incorporating the forces produced by electric fields on charged droplets we can steer individual droplets into selected channels; this provides a robust droplet-sorting module that allows us to select desired reaction products. These modules are the essential tools for high-speed manipulation and control of individual droplets and can serve as the enabling technology for droplet-based microfluidic devices. Moreover, because all control is carried out by switching electric fields, there are no moving parts and frequencies as high as  $10^5$  Hz are feasible; this facilitates very high throughput combinatorial technology.

We use soft lithography to pattern channels in polydimethylsiloxane (PDMS), a transparent polymer material.<sup>[26]</sup> A glass slide forms the top of the channel. We incorporate electric fields by patterning indium tin oxide (ITO) electrodes on the surface of the glass slide adjacent to the channels and seal the slide to the PDMS by using oxygen plasma. Devices fabricated in PDMS have the advantage of being strongly hydrophobic, which ensures that the oil carrier phase wets their surfaces and that the water droplets do not contact the channel walls, which facilitates the isolation of biomolecules and eliminates cross-contamination caused by surface interactions.

We use a flow-focusing geometry to form the drops. A water stream is infused from one channel through a narrow constriction; oil streams hydrodynamically focus the water stream and reduce its size as it passes through the constriction (Figure 1 A). This droplet generator can be operated in a flow regime that produces a steady stream of uniform droplets of



**Figure 1.** Charged-droplet generation. A) Oil and water streams converge at a 30-micron orifice. A voltage  $V$  applied to indium tin oxide (ITO) electrodes on the glass produces an electric field  $E$ , which capacitively charges the water–oil interface. The drop size is independent of charge at low field strengths but decreases at higher field strengths, as shown in the photomicrographs (scale bar: 100  $\mu\text{m}$ ): B)  $V=0$  V, C)  $V=400$  V, D)  $V=600$  V, E)  $V=800$  V. F) Droplet size as a function of voltage showing the crossover between flow-dominated and field-dominated droplet snap-off for three different flow rates of the continuous phase oil ( $Q_c=80$   $\text{nL s}^{-1}$  black; 110  $\text{nL s}^{-1}$  blue; and 140  $\text{nL s}^{-1}$  red). The infusion rate of the water is constant ( $Q_d=20$   $\text{nL s}^{-1}$ ).

water in oil. The size of the water droplets is controlled by the relative flow rates of the oil and the water; the viscous forces overcome surface tension to create uniform droplets. If the flow rate of the water is too high, a longer jet of fluid passes through the orifice and breaks up into droplets further downstream; these droplets are less uniform in size. If the flow rate of the water is too low, the droplet breakup in the orifice becomes irregular again, which produces a wider range of droplet sizes. Although this emulsification technology is robust, it is limited to producing droplets of one size at any given flow rate; this droplet size is largely determined by the channel dimensions. Moreover, the timing of the droplet production cannot be controlled.

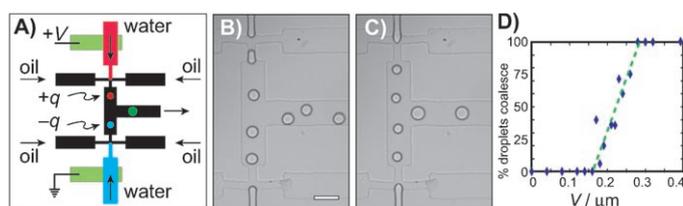
We overcome these limitations by incorporating electric fields to create an electrically addressable emulsification system. To achieve this, we apply high voltage to the aqueous stream and charge the oil–water interface, as shown schematically in Figure 1 A. The water stream behaves as a conductor whereas the oil is an insulator; electrochemical reactions charge the fluid interface like a capacitor. Upon droplet formation, the charge of the interface remains on the droplet. Furthermore, the droplet volume ( $V_d$ ) and frequency ( $f$ ) can be tailored over nearly three orders of magnitude without changing the infusion rate of the oil or the water. The size and frequency of the droplet are not independent; instead, their product is determined by the infusion rate of the dispersed phase  $Q_d=fV_d$ . The droplet size decreases with increasing field strength, as shown in Figure 1 B–E. The dependence of the droplet size on applied voltage for three different flow rates is summarized in Figure 1 F. The electric field has a negligible effect at low applied voltages, and droplet formation is driven exclusively by the competition between surface tension and viscous flow. By contrast, at high electric-field strengths, there is a significant additional force  $F$  on the growing drop given by  $F=qE$  where  $q$  is the charge on the droplet. Since the droplet interface behaves as a capacitor,  $q$  is proportional to the applied voltage  $V$ . This leads to a  $V^2$  dependence of  $F$ , which accounts for the decrease in droplet size with increasing applied field (Figure 1 F). If the electric field becomes too large, the charged interface of the water

stream is repelled by the highly charged drops; this destabilizes the production and increases the variation in droplet size.

The electronic control afforded by the field-induced droplet formation provides an additional valuable benefit: it allows the phase of the droplet break-off to be adjusted within the production cycle. This is accomplished by increasing the field above the critical break-off field only at the instant the droplet is required. This provides a convenient means to synchronize precisely the production and arrival of individual droplets at specific locations.

An essential component in any droplet-based reaction-confinement system is a mixer that combines two or more reagents to initiate a chemical reaction. Multicomponent droplets can easily be generated by bringing together streams of materials at the point where droplets are made.<sup>[27]</sup> However, all but the simplest reactions require multiple steps in which new reagents are added during each step. This can be accomplished by merging a new stream of fluid with large droplets passing the orifice;<sup>[28]</sup> however, this technique precludes the use of stabilizing surfactants, which limits its applicability when multiple processing steps require highly stable droplets. Instead, mixing can be best accomplished in droplet-based microfluidic reactors by combining different droplets, each containing individual reactants. However, this combination of droplets is particularly difficult to achieve in a microfluidic device because surface tension, surfactant stabilization, and drainage forces all hinder droplet coalescence;<sup>[29]</sup> moreover, the droplets must cross the stream lines that define their respective flows and must be perfectly synchronized to arrive at a precise location for coalescence.

The use of electrostatic charge overcomes these difficulties; placing charges of opposite sign on each droplet and applying an electric field forces them to coalesce. As an example we show a device consisting of two separate nozzles that generate droplets with different compositions and opposite charges (Figure 2A). The droplets are brought together at the confluence of the two streams. The electrodes used to charge the droplets upon formation also provide the electric field to force the droplets across the stream lines, thus leading to coalesce. Slight variations in the structure of the two nozzles result in slight differences in the frequency and phase of the droplet generation in the absence of a field. Thus the droplets differ in size even though the infusion rates are identical. Moreover, the droplets do not arrive at the point of confluence at exactly the same time. As a result the droplets do not coalesce, as shown in Figure 2B. By contrast, upon application of an electric field, the droplet formation becomes exactly synchronized, which ensures that pairs of identically sized droplets each reach the point of confluence simultaneously. Moreover, the droplets are oppositely charged, which forces them to traverse the stream lines and contact each other, thereby causing them to coalesce, as shown in Figure 2C. The remarkable synchronization of the droplet formation results from coupling of the break-off of each of the pair of droplets as mediated by the electric field; the magnitude of the electric field varies as the separation between the leading edges of the two droplets changes and the frequency of droplet break-off is mode-locked to the

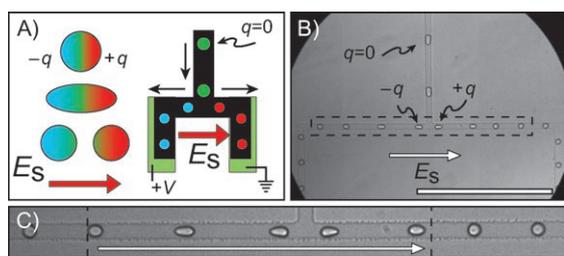


**Figure 2.** Coalescing drops. A) Drops having opposite sign of electrostatic charge can be generated by applying a voltage across the two aqueous streams. B) In the absence of an electric field the frequency and timing of drop formation at the two nozzles are independent and each nozzle produces a different-sized drop at a different frequency; the infusion rates are the same at both nozzles. After the confluence of the two streams, the drops from the upper and lower nozzles stay in their respective halves of the stream and, owing to the presence of surfactant, do not coalesce even in the case of large droplets that fill the width of the channel (scale bar: 100  $\mu\text{m}$ ). C) With an applied voltage of 200 V across the 500- $\mu\text{m}$  separation of the nozzles, the drops simultaneously break off from the two nozzles and are identical; simultaneous drop formation can be achieved for unequal infusion rates of the aqueous streams even up to a factor of two difference in volumes. D) The fraction of the drops that encounter each other and coalesce increases linearly above a critical field when the surfactant sorbitol monooleate (3%) is present.

electric field. A minimum charge is required to cause droplets to coalesce, presumably because of the stabilizing effects of the surfactant coating; this is evident from Figure 2D, which shows the voltage dependence of the percentage of drops that contact each other that actually coalesce.

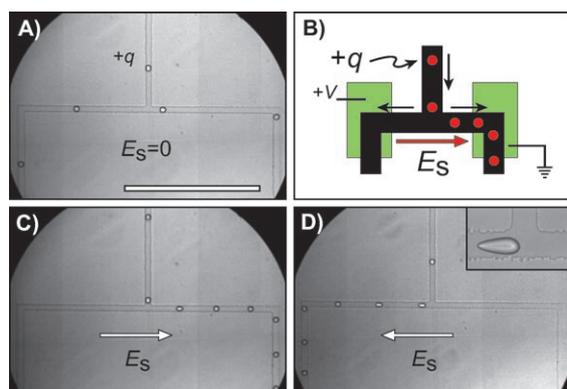
The use of oppositely charged droplets and an electric field to combine and mix reagents is an extremely robust method, and 100% of the droplets coalesce with their partner from the opposite stream. However, after they coalesce the resultant drops carry no electrostatic charge. Although it is convenient to charge droplets during formation, other methods must be employed in any robust droplet-based microfluidic system to recharge the mixed droplets if necessary for further processing. This is readily accomplished through the use of extensional flow to split neutral droplets in the presence of an electric field, which polarizes them and results in two oppositely charged daughter droplets (Figure 3A). The photomicrograph in Figure 3B shows neutral droplets entering a bifurcation and splitting into charged daughter droplets. The dashed region in Figure 3B is enlarged in Figure 3C to illustrate the asymmetric stretching of the charged droplets in the electric field. The vertical dashed lines indicate the edges of the electrodes, where the droplets return to their symmetric spherical shape. The electric field also allows precision control of the droplet splitting and provides the basis for a robust droplet-division module that allows the splitting of the contents into two or more aliquots of identical reagent, which could facilitate multiple assays on the contents of the same microreactor.

A final component essential for the construction of microfluidic droplet-reaction systems is a droplet sorter. The contents of individual droplets must be probed, and selected droplets sorted into discreet streams. Such sorting in microfluidic devices can be accomplished through the use of mechanical valves.<sup>[16]</sup> Droplets can be passively sorted by size,



**Figure 3.** Recharging neutral drops. A) Neutral drops are recharged by breaking them apart in the presence of an electric field. Uncharged drops ( $q=0$ ) are polarized in an electric field ( $E_s \neq 0$ ), and provided  $E_s$  is sufficiently large, as shown in the photomicrograph of (B; scale bar:  $500 \mu\text{m}$ ), they break into two oppositely charged daughter drops in the extensional flow at a bifurcation. The enlargement of the dashed rectangle, shown in (C), reveals that the charged drops are stretched in the electric field but become spherical again upon contact with the electrodes indicated by dashed vertical lines.

through creative use of flow geometry;<sup>[20]</sup> however, sorting is most useful when based on content rather than size. The use of electrostatic charging of droplets provides an alternate means that can be precisely controlled, can be switched at high frequencies, and requires no moving parts. The electrostatic charge on the droplets enables drop-by-drop sorting based on the linear coupling of charge to an external electric field. As an example, a T-junction bifurcation that splits the flow of carrier fluid equally will also randomly split the droplet population equally into the two streams, as shown in Figure 4A. However, a small electric field applied at the bifurcation precisely dictates which channel the drops enter; a schematic of the electrode configuration is shown in Figure 4B. Varying the direction of the field varies the direction of the sorted droplets as shown in Figure 4C and D. The large forces that can be imparted on the droplets and the high switching frequency make this a fast and robust sorting engine



**Figure 4.** Manipulating charged drops. A) Charged drops alternately enter the right and left channels when no field is applied ( $E_s=0$ ; scale bar:  $500 \mu\text{m}$ ). B) Layout for using an electric field  $E_s$  to select the channel that charged drops will enter at a bifurcation. C) When an electric field is applied to the right, the drops enter the right branch at the bifurcation. D) The drops enter the left branch when the field is reversed. After the bifurcation, the distance between drops is reduced to half of what it was before, which indicates that the oil stream is evenly divided. The inset of (D) shows the deformation in the shape of a highly charged drop in an electric field.

with no moving parts; thus the processing rate is limited only by the rate of droplet generation.

The enhanced functionality that electrostatic charge brings to droplets in microfluidic devices has the potential to enable an expansive list of microfluidics applications. This toolkit of techniques for manipulating droplets will enable modular integration of systems for transporting and carrying out reaction of small numbers of molecules. High-throughput screening, combinatorial chemistry, and the search for rare biological function in libraries of biomolecules could all potentially benefit from electrostatic manipulation of droplets in microchannels. Droplet-based microfluidic technology can also be used to develop a chip-scale fluorescence-activated cell sorter (FACS) with enhanced activation functionality that goes beyond fluorescence to include multiple reagent-based assays between the droplet formation and sorting steps. Moreover by using femtoliter droplets, which are a few microns in diameter, even a single biomolecule represents a concentrations of approximately  $1 \text{ nM}$ , which is sufficient for efficient chemical reactivity and single-molecule assays.<sup>[30]</sup>

Many of the potential uses of droplet-based microfluidic devices are driven by a need to encapsulate a varied population or library of molecules, cells, or particles into microreactors, perform an assay on the contents, perhaps through the addition of reagents, and then, finally, to remove selectively specific microreactors from the collection in a search for rare events. This requires processing rates of approximately  $10^3$  per second to sort through the smallest libraries in a reasonable time whereas rates on the order of  $10^5$  per second are desirable for larger libraries. These rates are feasible with the charged-droplet paradigm. Moreover, because the microfluidic devices are stamped, parallel flow streams can be fabricated, further enhancing the total throughput. Combined, the advantages of droplets and high-throughput manipulation provide significant opportunity for widespread application. The platform technology presented herein will facilitate the potential application of droplet-based microfluidic technology.

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