

# Chapter 2

## Microfluidic Droplet Manipulations and Their Applications

Melinda G. Simon and Abraham P. Lee

### 2.1 Introduction

“Droplet microfluidics” enables the manipulation of discrete fluid packets in the form of microdroplets that provide numerous benefits for conducting biological and chemical assays. Among these benefits are a large reduction in the volume of reagent required for assays, the size of sample required, and the size of the equipment itself. Such technology also enhances the speed of biological and chemical assays by reducing the volumes over which processes such as heating, diffusion, and convective mixing occur. Once the droplets are generated, carefully designed droplet operations allow for the multiplexing of a large number of droplets to enable large-scale complex biological and chemical assays. In this chapter, four major unit operations in droplets are discussed: droplet fusion, droplet fission, mixing in droplets, and droplet sorting. Combined, these operations allow for much complexity in executing chemical reactions and biological assays at the microscale. A broad overview of potential applications for such technology is provided throughout. While much research effort has been focused on the development of these individual devices, far fewer attempts to integrate these components have been undertaken. A review of many microfluidic unit operation devices is provided here, along with the advantages and disadvantages of each approach for various applications.

---

M.G. Simon • A.P. Lee (✉)

Department of Biomedical Engineering, University of California-Irvine, Irvine, CA, USA

e-mail: [aplee@uci.edu](mailto:aplee@uci.edu)

## 2.2 Droplet Fusion

### 2.2.1 Theory

Droplet fusion is a critical operation for droplet manipulation, since it allows for the combination of different reagents and for the initiation of chemical and biological reactions in microfluidic devices. Contrary to intuition, simply initiating droplet collisions does not frequently result in fusion between the droplets. In fact, a systematic study of a passive droplet fusion technique revealed that it is the separation process of closely spaced microdroplets, rather than their collision, which results in coalescence of the droplets [1]. Bibette et al. provide a set of equations for predicting coalescence. For coalescence of droplets to occur, the continuous phase separating the two droplets must first be drained, bringing the droplets into close contact. Then, the droplets must be kept in close contact for a critical minimum amount of time, in order for fusion to occur. Fusion occurs due to fluctuations in the surface tension on the surface of droplets, which cause destabilization of the interface between the oil and water phases [2].

Although the fusion of droplets may seem straightforward, there are several key challenges involved in this process. In order for droplets to fuse, they must achieve temporal and spatial synchronization. Several creative strategies have been employed to synchronize droplets prior to fusion, both for passive and active droplet fusion systems. Still, with the development of more complex microfluidic systems with a large number of inputs, new strategies for the synchronization of droplets are being sought. The addition of surfactant to either the continuous or dispersed phases of a droplet microfluidic device is a common practice to stabilize the droplets; however, the presence of surfactant makes droplet fusion much more difficult. Other important considerations for any droplet fusion mechanism are its throughput and efficiency. While some methods presented below demonstrate a very high efficiency of fusion, with the vast majority of droplet pairs undergoing fusion, the throughput of such systems may be much lower than a system where the efficiency of fusion is not quite as high. While both high fusion efficiency and high throughput are desirable, it may be necessary to compromise one or the other of these qualities in order to satisfy the demands of the intended application. Due to the fact that fusion involves the coming together of contents from different droplets, inter-droplet contamination is also a concern. Additionally, preservation of the viability of biological material may be a concern in active fusion methods where electricity is used to fuse droplets. While passive fusion methods often carry a lower risk of contamination and are more biocompatible, they generally have a much lower throughput than active fusion methods. As a result, a variety of both passive and active methods for inducing the fusion of droplets have been developed. While each design has its strengths and shortcomings, a suitable method for inducing droplet fusion may certainly be found for a variety of applications.

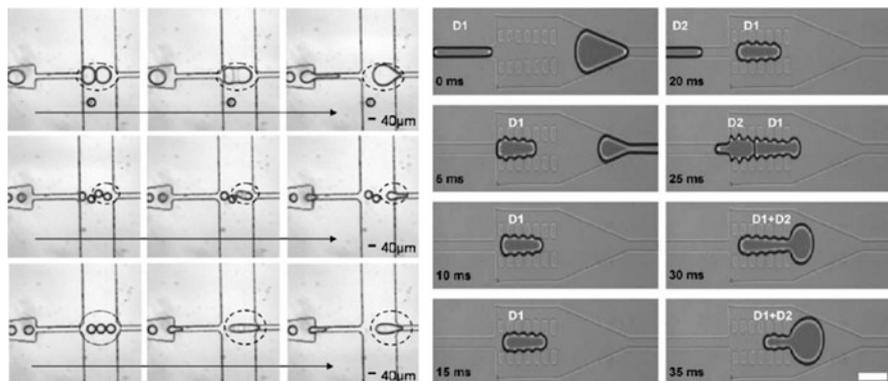
## 2.2.2 *Passive Fusion Methods*

Passive droplet fusion mechanisms are those which do not require active control or electricity. These designs are often simpler than active fusion mechanisms, which may require complicated circuitry and control systems. One advantage of most passive droplet fusion techniques is that the possibility of inter-droplet contamination is lower than for active droplet fusion techniques. However, passive droplet fusion techniques are limited by the rate at which natural phenomena, such as surface tension fluctuations occur, and are therefore often slower than most active droplet fusion techniques.

### 2.2.2.1 *Geometry-Mediated Passive Fusion*

Among the earliest and simplest designs for passive fusion of microdroplets employs a section of widened channel, termed an “expansion volume.” By controlling the continuous phase velocity and the dimensions of the expansion volume, consecutive droplets can be induced to fuse in this region. The expansion volume enables fusion by draining the continuous phase that separates one or more consecutive droplets in a channel. Upon entering the expansion volume, the continuous phase fluid spreads around the droplets to fill the increased volume, while droplets remain in the center of the channel. This removal of spacing between droplets allows interaction between the surfaces of adjacent droplets and induces fusion of the droplets as a result of minute disturbances in surface tension. Either a gradually tapering channel [3] or a wider section of the channel [4, 5] may be employed as an expansion volume (Fig. 2.1). Careful control of the frequencies of droplet generation from each droplet source is necessary for reliable fusion when using an expansion volume [6]. Another early method for passively inducing the fusion of consecutive droplets involved active drainage of the continuous phase between droplets, known as a flow-rectifying design. In this design, the droplet stream would flow past a junction with channels perpendicular to the direction of droplet transport. Through these perpendicular channels, continuous phase could be actively removed using an off-chip syringe pump, which induced droplet fusion by bringing consecutive droplets close together [4, 7, 8]. Alternatively, a 3D expansion volume can be used to fuse droplets. In this design, droplets carried along a microchannel “track” are carried through a chamber of larger width and height than the track. A control line adds continuous phase at a prescribed rate to control the number of fusion events that occur in the chamber. By controlling the flow rate of continuous phase from this line, researchers were able to perform arithmetic operations with the droplets, similar to the operation of an abacus [9].

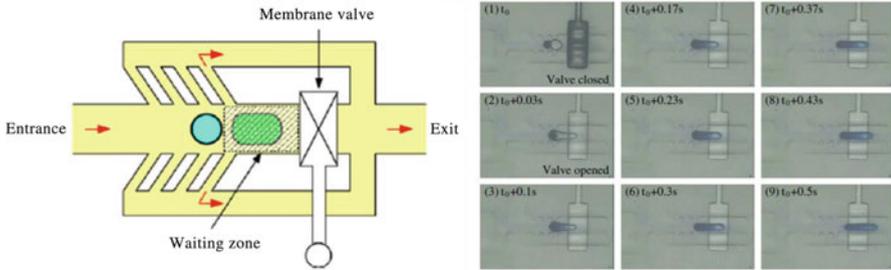
Using an expansion volume, droplets from two different inlets have been fused in a tapering region of a microfluidic device. In this device, alternating droplets from two different inlets were produced. The droplet pairs fuse downstream in a tapering region of the channel to yield CdS nanoparticles within the



**Fig. 2.1** *Left:* As droplets enter an expansion of the main fluidic chamber, the continuous phase is drained from between them and droplet fusion occurs (Reproduced from Tan et al. [4], by permission of the Royal Society of Chemistry, <http://dx.doi.org/10.1039/b403280m>) *(right)* Niu et al. used a pillar array to slow and trap a droplet entering an expansion of the main fluidic channel. The first droplet remains in the trap until a second droplet enters. After continuous phase drains from between them, the two droplets fuse, and hydrodynamic pressure from the entering second droplet pushes the fused droplet out of the trap and downstream (Reproduced from Niu et al. [10], by permission of the Royal Society of Chemistry, <http://dx.doi.org/10.1039/b813325e>)

fused droplets [3]. This development enabled the execution of simple chemical reactions, comprising a single mixing step, on a chip. The microfluidic platform enables synthesis of very small volumes of product, useful in situations where the reactants are expensive, hazardous, or simply limited.

While an expansion volume provides a way to fuse droplets passively, there are several limitations to this design. With the expansion volume approach, only consecutive droplets in a channel can fuse. This restriction requires that the order of droplets in a microchannel be carefully controlled, in order to achieve the desired fusion. In addition, an expansion volume is only able to fuse droplets that have a relatively small and uniform inter-droplet spacing. In order to enable more complex applications involving droplet fusion, several devices have been developed which overcome the problem of inter-droplet spacing, to ensure reliable droplet fusion. Building from the expansion volume concept for droplet fusion, Niu et al. designed an expansion volume containing two sets of tapering pillars in its center (Fig. 2.1). As a droplet enters the expansion volume, it is squeezed between the sets of pillars. Once the droplet has completely entered the expansion volume, continuous phase behind the droplet is allowed to drain around the droplet into the widened channel. The droplet is stopped in the pillar array due to the increase in surface tension it experiences. As the pillars narrow, the radius at the front of the droplet becomes smaller than the radius at the back of the droplet, which produces a net surface tension pressure that counters the hydrostatic pressure induced by the continuous phase. In this device, a droplet can be held indefinitely until the next droplet in the line approaches—this feature allows for droplet fusion to occur even if inter-droplet spacing does not remain constant. In addition, by changing the size of the expansion



**Fig. 2.2** A membrane valve and continuous phase drainage channels allow the first droplet to slow and become trapped. As a second droplet approaches the first and slows, the valve is released and the two droplets are fused as they leave the trap (Reproduced from Lin and Su [12], with permission from IOP Publishing, Ltd.)

chamber and the size of the droplets, multiple droplets can be induced to fuse in the pillar array [10]. In another design, droplets from one inlet become trapped in a narrowing channel, due to the increase in surface tension they experience as the radius of the front of the droplet decreases. Once a droplet is trapped, it is held in place indefinitely by surface tension forces. A bypass channel allows continuous phase and other droplets to continue to flow around the trapped droplet. Another droplet inlet, perpendicular to the first inlet, joins a fusion chamber at the location where a droplet is trapped. As a droplet from this second inlet approaches the fusion chamber, the second droplet fuses with the trapped droplet as it passes by. Due to the ability of this device to trap the first droplet and hold it indefinitely, droplets from separate inlets, and generated at different respective frequencies, can be fused. In addition, the inter-droplet spacing need not be uniform or short, for fusion to occur [11].

Instead of using surface tension forces to trap droplets before fusing, a membrane valve has also been used (Fig. 2.2). For fusion to occur, a membrane that occludes most of the width of an expansion volume is depressed. Droplets are constrained to the center of the expansion volume by a set of pillars as the membrane is depressed, while continuous phase is allowed to flow around the pillars and the depressed membrane. Once the desired number of droplets has been trapped in the expansion volume, the membrane valve is opened [12]. Droplets fuse as they are pulled away from one another out of the expansion volume. This is in accordance with recent theory and characterization describing how droplets are observed to merge as they are moving away from one another, instead of when they are pushed together [1].

### 2.2.2.2 Passive Fusion Induced by Physical and Chemical Phenomena

In addition to the above methods for passive droplet fusion, several designs which exploit physical or chemical phenomena have been developed. For instance,

droplets of different sizes or of different viscosity travel at different speeds within microfluidic channels. If a small droplet is introduced to the channel after a larger droplet, the small droplet will travel faster in the channel than the larger droplet, which decreases the inter-droplet spacing and allows fusion to occur at an expansion volume. As the viscosity of the dispersed phase is increased, the droplets travel with slower velocity. Hence, a droplet of lower viscosity will travel faster through a microchannel than a droplet of higher viscosity, allowing the lower viscosity droplet to “catch up” to a higher viscosity droplet. Droplets paired in this way can then become merged when they enter an expansion volume. One advantage to this technique is that droplets can self-synchronize if conditions are right, eliminating the need for an active mechanism of pairing droplets [13].

Surfactants are often added to a microdroplet emulsion to stabilize the droplets and prevent unwanted fusion events. One group has exploited the readiness with which surfactant-free droplets fuse to reliably fuse two populations of droplets one-to-one. A population of droplets with a surfactant concentration of 2.8% was generated and injected into a chip, where each surfactant-stabilized droplet was paired with a surfactant-free droplet. Upon entering a microchannel with 117° bends, the droplet pairs readily fused. Fusion occurs as a result of the geometric constraints imposed by the zigzag channel as well as the partial instability of the surfactant-free droplets. Since the droplets were paired one-to-one, secondary fusion of droplets was avoided, as fused droplets all contained surfactant and were thus stabilized against further fusion. Although useful for one-to-one fusion of two different droplet populations, this technique requires careful consideration of the chemistry of the system. Furthermore, the relatively high surfactant concentration required for the surfactant-stabilized population of droplets may be incompatible with some chemical or biological assays [14].

Another method which does not require synchronization of droplets for fusion relies on a hydrophilic patch inside a microchannel to trap droplets before fusion. A photomask is used to allow selective polymerization of acrylic acid on a PDMS device, using UV light. Areas exposed to the UV light are patterned with polyacrylic acid, rendering these areas hydrophilic. The rest of the PDMS device retains its native hydrophobicity. When a hydrophilic droplet approaches the hydrophilic patch in the channel, the droplet is slowed and stopped over the patch. Additional droplets gather behind the first droplet near the patch, until the viscous drag force generated by their presence is enough to overcome the interfacial forces holding the first droplet to the patch. At this point, the first droplet begins to move downstream, and the trapped droplets all fuse. Since this device functions by balancing the interfacial interaction force between the droplets and the patch, and the viscous drag force imposed by the continuous phase, the number of droplets to be trapped and fuse can be tuned by changing the continuous phase flow rate, which changes the viscous drag force on the droplets [15]. Although this fusion mechanism requires no special geometry and could potentially be incorporated into any straight microchannel, the possibility for contamination between droplets exists, due to the requisite interaction of droplets with the polyacrylic acid patch in the channel.

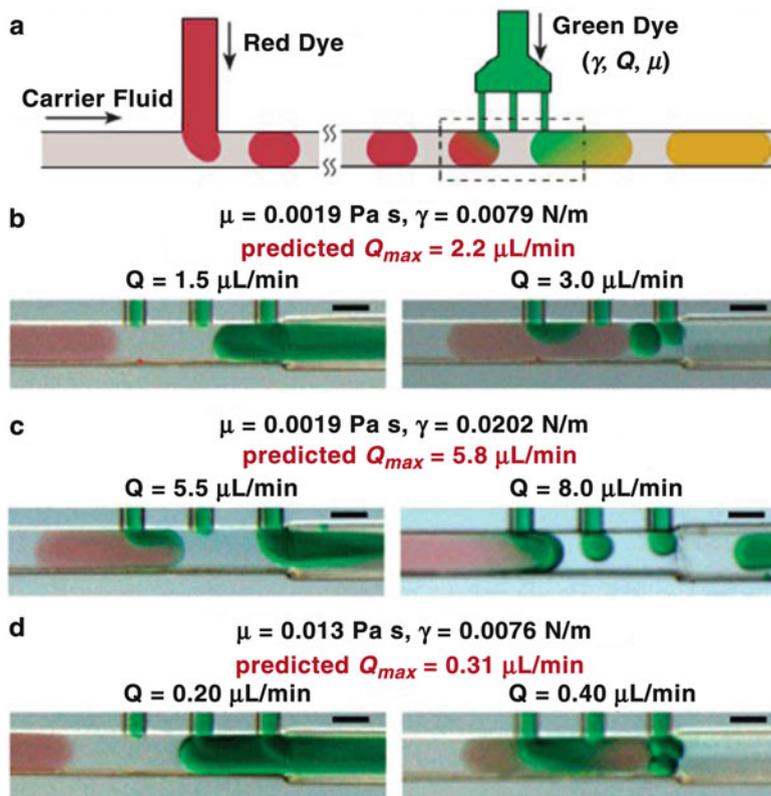
A unique approach to droplet fusion using a laser has been attempted in recent years. By carefully directing an Argon ion laser in a microchannel, localized heating is induced in the channel which can induce fusion between two droplets. Droplet fusion is induced when the laser is directed to the interface between the two droplets. Heat applied at this location leads to disturbances in the surface tension which result in destabilization of the droplet interfaces and eventually fusion. The laser is also capable of stopping droplets in a channel, potentially allowing for trapping a given number of droplets and fusing them together, using only a laser. Although this design minimizes the chance of inter-droplet contamination, due to the fact that droplets are not physically constrained on a surface, the throughput of this approach may be lower due to the need to precisely control the timing and location of laser heating [16].

### 2.2.2.3 Adding Reagents into Passing Droplets

An alternative to passive droplet fusion schemes that generate two droplets and then fuse them together is a technique where reagent is metered into a passing droplet from a microchannel intersecting the main channel. This strategy accomplishes the same goal of droplet fusion and precludes the need to generate many different populations of droplets. This technique has been used to produce nanoparticles in microdevices that are more monodispersed than nanoparticles produced in a benchtop process [17]. One drawback to this technique is that the possibility for contamination is more significant, since passing droplets come into direct contact with the second reagent stream. To overcome this issue, a device has been introduced more recently that uses several narrow hydrophilic channels to introduce the second reagent (Fig. 2.3). This reduction in the dimension of the injection channel raises the dimensionless Peclet number in this design, meaning that addition of reagent to passing droplets is due more to convection than diffusion. The authors postulate that the smaller injection channel dimension minimizes the effect of diffusion, which causes contamination between droplets due to the chaotic mixing it introduces. Although temporal synchronization of the release of the second reagent was a problem in the earlier designs, the technique employing several narrow channels can avoid the problem of extra droplet formation by carefully selecting the volumetric flow rate of the continuous and dispersed phases [18]. One disadvantage to this approach is less control over the specific amount of reagent that is added to a passing droplet. In systems where two separate droplets are generated and then brought together, the calculation of the specific volume of added reagent is more straightforward.

### 2.2.3 Active Fusion Methods

In addition to the passive fusion techniques discussed above, other fusion methods employing active controls, such as electrocoalescence, dielectrophoresis,



**Fig. 2.3** As an alternative to fusing droplets, reagent may be metered into passing droplets from several narrow side channels. Successful injection of substrate requires a careful balance of the volumetric flow rates of the continuous and dispersed phases (Reproduced with permission from Li et al. [18])

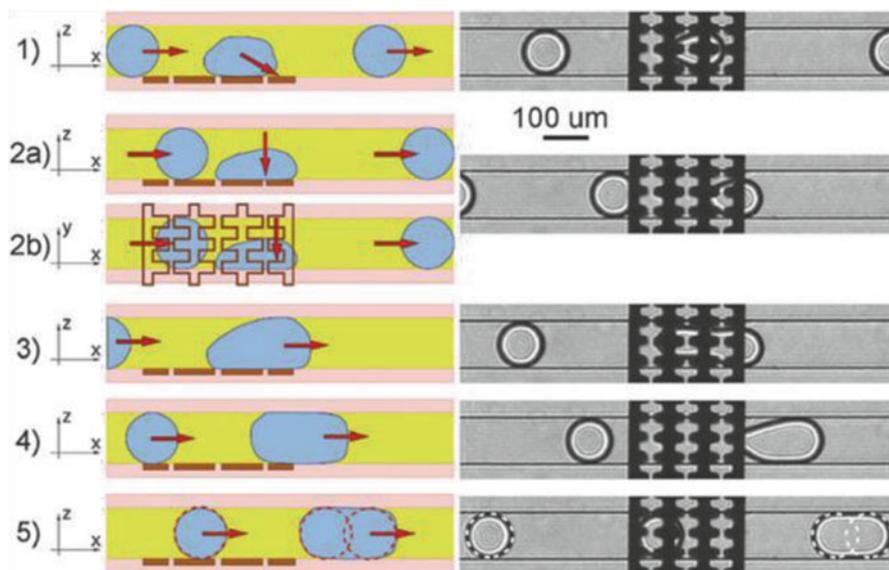
and optical tweezers have also been developed. Such methods are inherently more complex than many passive droplet fusion schemes, since many require fabrication of electrodes and precise timing of electrical signals in order to fuse droplets. With the use of electricity come concerns of contamination between droplets, if some of the droplet contents become deposited on an electrode, as well as biocompatibility of electrical signals on biological molecules, such as DNA or proteins. The advantage to such systems is that the use of electricity can hasten the development of instabilities in the surface tension between droplets [19], initiating fusion more quickly and increasing the throughput capabilities of the device.

While several research groups have developed devices to fuse microdroplets using electrodes, the size and positioning of the electrodes in these devices is diverse, with each design presenting its own strengths for particular applications. In one design with applications for studying chemical kinetics, the electrodes comprise a platinum wire that is positioned inside the main microfluidic channel

where continuous phase flows, and an indium tin oxide ground electrode on the base of the microfluidic device. Perpendicular to this main channel, two dispersed phase streams enter near the wire and form droplets through a simple T-junction configuration. When voltage is applied across the electrodes, fusion of one droplet entering from each of the dispersed phase channels occurs at the tip of the wire. The application of voltage in this nonuniform electric field induces a positive dielectrophoretic (DEP) force on the droplets, which pulls them toward the wire. Once both droplets have been pulled close to the wire, the layer of continuous phase separating them becomes thin, and instabilities in the surface tension between the droplets result in fusion. Using this device, the progress of a chemical reaction can be tracked optically in the droplets, which each act as individual microreactors. Since the rate of droplet formation in the device is constant, the droplets produced display the progress of the chemical reaction at discrete time points, providing a simple means of studying the kinetics of a reaction [20]. While well suited for this purpose, this device is limiting in that the contents of all the fused droplets are exactly the same. For many applications, the production of many droplets with diverse contents is necessary.

For more complicated reactions or assays, the fusion of multiple droplets may be desired or needed. In 2009, Tan et al. developed a round microfluidic chamber in which a variable number of droplets could be fused. The chamber is designed to slow entering droplets, to give them more time for fusion, and helps to position the droplets parallel to the electric field. This orientation minimizes the electric field strength needed for droplet fusion, which occurs when the electric field disrupts surfactant molecules on the surface of the droplets. The fusion chamber is also designed large enough that droplets do not contact the electrodes during the fusion process, which decreases the risk of droplet-to-droplet contamination that may occur in devices where droplets come into contact with the electrodes. For fusion of two droplets, only the electric field is necessary both to align the droplets correctly and to fuse them. To fuse several droplets, however, laser tweezers were employed to position all of the droplets in a line parallel to the electrical field. The disadvantage to this technique is its low throughput. Five 10  $\mu$ s pulses of DC voltage, spaced 0.2 s apart, were required for a single fusion event [21]. Due to the spaces between pulses, the maximum rate of fusion would be less than one event per second, which is much slower than most other droplet fusion mechanisms.

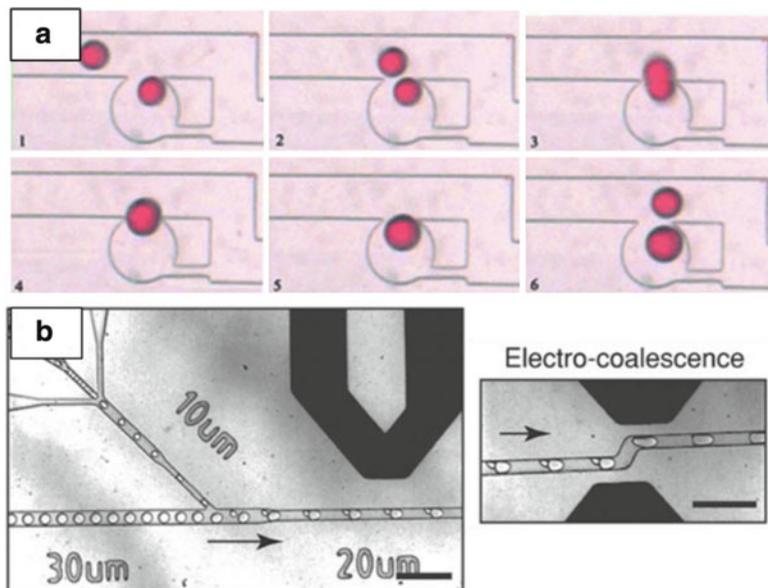
Another device traps passing droplets on the electrode surface to induce the fusion of multiple droplets. As the droplet slows and becomes trapped, it deforms and spreads on the electrode surface, which provides space allowing continuous phase to flow around the droplet. The next droplets carried through the channel also become trapped on top of the electrodes and fuse with previously trapped droplets (Fig. 2.4). The ability of the electrodes to trap and hold the droplets on their surface is a balance achieved between the DEP force imposed by the electrodes and the hydrodynamic force imposed by the flow of the continuous phase in the channel. Eventually, as multiple droplets become trapped on the electrodes, the hydrodynamic force on the droplets overcomes the DEP force from the electrodes, and the fused droplet is released from the electrode surface. The number of droplets to



**Fig. 2.4** Electrodes are used to slow and trap a droplet. Deformation of the droplet onto the electrodes allows continuous phase to pass around the droplet. As a second droplet approaches, it fuses with the first, and the blockage of continuous phase flow produces force that pushes the fused droplet off of the electrodes (Reproduced from Zagnoni and Cooper [19], by permission of The Royal Society of Chemistry, <http://dx.doi.org/10.1039/b906298j>)

be fused on the electrodes can thus be controlled by careful selection of the DEP force and hydrodynamic pressure applied to the system. Fusion rates of 50 per second were routinely demonstrated with this device. Higher rates of up to 100 fusions per second were achieved, but at high voltage levels, which could provide problems for biological assays and can also induce hydrolysis of water in the droplets. In addition, the reliance of this technique on droplet contact with the electrodes could mean that contamination from droplet-to-droplet is likely. One advantage to this technique is that no synchronization system is required. The first trapped droplet can wait indefinitely on the electrode surface until subsequent droplets arrive [19].

A common shortcoming in active fusion designs is their incompatibility to accommodate biological solutions, owing to the high voltages applied to induce droplet fusion. To address this problem, Priest et al. demonstrated a device that requires only a 1 V DC pulse to fuse droplets—a considerably lower voltage than many other systems. Lower voltages are required in this system due to the proximity of the droplets to the electrodes. While other designs used an expansion chamber to fuse their droplets, necessitating high voltage values to span a larger area [21], these design droplets are tightly packed as they flow past the electrodes. Even with tightly packed droplets, isolated fusion events between adjacent droplets can occur, as long as the droplet interface where fusion takes place is parallel to the electrodes.



**Fig. 2.5** (a) Droplets may be stored and fused in the same device by applying a DC voltage across the fluidic channel. Here, fused droplets may be removed from the array by increasing the continuous phase flow rate (Reproduced from Wang et al. [23], by permission of The Royal Society of Chemistry, <http://dx.doi.org/10.1039/b903468d>). (b) Droplets are paired and fused by an AC field (Reproduced from Mazutis et al. [29], by permission of The Royal Society of Chemistry, <http://dx.doi.org/10.1039/b907753g>)

For different types of droplet packing, different electrode orientations could be used to achieve this purpose. Fusion rates of around 10 per second can be obtained using this technique. In addition to the increased biocompatibility of this approach, an insulating layer of Poly(methyl methacrylate) coats the electrodes in the device, which reduces the chance of contamination, another significant concern for active fusion devices [22].

One unique approach to active droplet fusion allows for the trapping and storage of an array of fused droplets, in order to observe a reaction or the behavior of a cell over time. The first droplet is trapped in a side compartment adjoining the main fluidic channel when a DC voltage is applied (Fig. 2.5a). Voltage, applied across the channel, induces a DEP force on the droplet that causes it to move in one direction or another, depending on the type of DEP force applied. Using the DC voltage again, this trapped droplet can be induced to move toward the main channel, where it can contact and fuse with a passing droplet. Once fused, the droplet returns to the side compartment as the DC voltage is switched off, and the droplet may be observed indefinitely in the side compartment. These structures are designed to keep the fused droplet trapped at lower continuous phase flow rates, but allow the compartments to be cleared when the continuous phase flow rate is increased [23].

Instead of using electrodes to induce coalescence at the point of droplet contact, an alternative design imposes an opposite electrical charge on different populations of droplets, which then become fused together in the presence of an electrical field. As droplets are generated, either a positive or negative charge is imposed on them, such that droplets from one inlet become positively charged, while droplets from a second inlet become negatively charged. A potential disadvantage to this approach is that fused droplets become electrically neutral. In order to perform more than one fusion step, droplets would need to undergo charging after each fusion event [24].

Expanding upon a concept used to fuse droplets passively, one active fusion scheme induces pairing of droplets prior to fusion by generating droplets of different sizes. Smaller droplets move more rapidly through microfluidic channels, which cause them to catch up to and pair with larger droplets. Once paired, the droplets are fused controllably by a pair of electrodes across the microfluidic channel [25].

Electrowetting is another approach that has been used to manipulate droplets, inducing droplet formation as well as fusion. In this approach, droplets are positioned atop an array of individually addressable electrodes, and deform over the electrode when a voltage is applied, due to a minimum in the electric field that is induced over the electrode. Using computer software, the electrodes can be activated in a certain order to induce movement of the droplet. To fuse droplets using this technique, two droplets need only to be brought into close proximity using the electrode array. Although this technique offers very precise control over the movement of droplets, inter-droplet contamination is a concern, since the droplets wet the surface of the electrodes when they are trapped [26].

For most applications, droplet fusion is desired to initiate a chemical reaction; however, it may be necessary for some applications to convert information contained in individual droplets into a continuous stream for the purpose of analysis of droplet contents. For this purpose, Fidalgo et al. designed a device whereby selected droplets in a stream of oil could be induced to merge with an adjacent aqueous stream. If a droplet is selected to merge with the aqueous stream, an electric field is applied, inducing a DEP force on the droplet which causes it to move into the aqueous stream [27].

One particularly exciting application for droplet fusion technology is the ability to fuse liposomes or cells. Using a device with embedded electrodes, liposomes and prokaryotic cells were fused using a device which applied alternately AC and DC voltages. First, AC voltage is applied to align the liposomes or cells for fusion. This alignment is followed by the application of a DC voltage which fuses the liposomes or cells. Although this technique could find wide application in a wide variety of studies on cellular gene regulation, the fusion rate is relatively low at 75%, and the throughput of the technique is also very low, requiring 5 s alone to position liposomes close together, and another full second for the fusion event to occur [28].

Recently, electrofusion has also been used to combine reagents for the study of the kinetics of a biological reaction, such as the activity of the translated protein of the *cotA* laccase gene. Using electrodes on either side of the microfluidic channel, AC voltage was applied at a frequency of 30 kHz. Fusion events occurred at a rate

of 3,000 per second, and a high fusion efficiency of 90% was achieved (Fig. 2.5b). This high fusion rate provided by this active droplet fusion scheme allows a more precise study of the kinetics of the reaction, since more samples are produced with a shorter time step between them than could be achieved using a passive droplet fusion device [29].

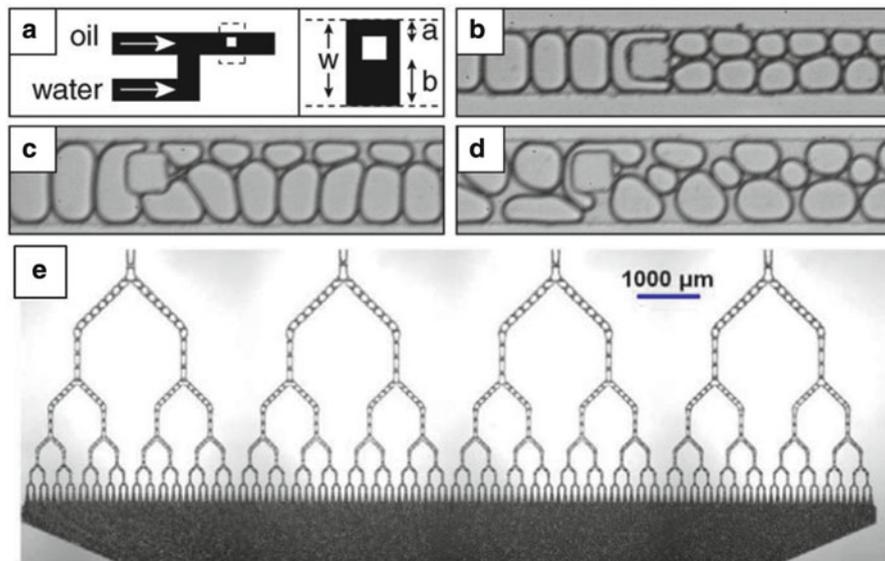
Electrical fields may also be used to introduce reagent into passing droplets, as demonstrated by Abate et al. A series of fluid-dispensing channels were oriented perpendicular to a main microfluidic channel, and each dispensing channel contained a set of electrodes. By momentarily applying an electrical field across a set of electrodes, reagent could be dispensed from that channel into droplets in the main microfluidic channel without the use of valves. The application of an electrical field also destabilizes the interface between phases, leading to fusion of the passing droplet with the injected reagent. The amount of fluid injected into passing droplets may be tuned by adjusting the pressure in the dispensing channel as well as the velocity of the passing droplets. Using this device, researchers were able to add reagent selectively into passing droplets at a rate of 10,000 droplets per second [30].

## 2.3 Droplet Fission

The fusion or combining of the contents of different droplets has obvious importance for the execution of chemical reactions in droplet microfluidic systems; however, the ability to divide droplets is also a necessary operation for the execution of assays and the production of sample replicates. A simple way to introduce multiple sample types into a microfluidic device involves the consecutive aspiration of a large plug of fluid from a number of microtiter plate wells. In these devices, droplet fission is used to divide the plug into many smaller volume droplets, which allow individual droplets to be paired and mixed with different reagents [31], and also provide a smaller volume container, which allows for rapid mixing and reduces the reaction time in the droplet [32]. Droplet fission designs also provide the potential to increase the throughput of droplet production, and to digitize biological assays, increasing their sensitivity. Like droplet fusion, both passive, geometry-mediated droplet fission schemes have been developed, along with active droplet fission schemes, which employ the use of electricity or localized heating to split droplets.

### 2.3.1 *Geometry-Mediated Splitting*

Link et al. developed two simple methods to induce droplet fission, using only the geometry of the microchannels. In one device, a simple bifurcation of the main microfluidic channel is introduced in order to split droplets. Through experimentation, it was determined that droplet fission would occur at this bifurcation if the droplet is plug-like: that is, when the length of the droplet in the microchannel is



**Fig. 2.6** (a–d) A large post structure in a microchannel packed with droplets mediates droplet splitting (b) symmetrically and (c, d) asymmetrically, depending on the placement of the post (Reproduced from Link et al. [33], Copyright 2004 by the American Physical Society, <http://prl.aps.org/abstract/PRL/v92/i5/e054503>). (e) Repeated fission is induced by  $45^\circ$  bifurcation junctions (Reproduced with permission from Hsieh, et al. [35])

greater than the circumference on the edge of the droplet. The droplet splits evenly if the resistances of the two daughter channels—downstream of the bifurcation—have the same fluidic resistance. Since fluidic resistance is proportional to microchannel length, according to the Hagen–Poiseuille equation, changing the length of one of the two daughter channels allows droplets to split unevenly. In this way, the volume ratio of the daughter droplets produced by the fission can be changed. Another design proposed by the group employed a large post near the middle of a microfluidic channel to induce droplet fission (Fig. 2.6a–d). By adjusting the position of this large post in the microchannel, the ratio of sizes of daughter droplets can be changed [33].

Building on this technology, another group used a repeating bifurcation structure to split a single parent droplet into 8 or 16 daughter droplets of nanoliter volumes. The bifurcation structure consists of a T-shaped junction, where the parent microfluidic channel meets the daughter microfluidic channels at an angle of  $90^\circ$ . This group observed asymmetric splitting of droplets despite symmetric channel designs, in devices containing consecutive bifurcations. It was hypothesized that asymmetric droplet breakup was due to a high surface tension pressure relative to the pressure drop in the microchannel. They determined that asymmetric splitting in bifurcating junctions can be minimized by keeping the surface tension low, for example, by adding surfactants to the system, or increasing the flow rate through the device [34].

Utilizing a different bifurcation design, a single droplet was split into 128 monodispersed droplets. In this design, the parent microfluidic channel splits into daughter channels at an angle of  $45^\circ$  forming a Y-shaped bifurcation junction (Fig. 2.6e). Hsieh et al. found that using a bifurcation channel angle of  $45^\circ$  reduced the asymmetric breakage of droplets, when compared to a design using an angle of  $90^\circ$ . In addition, the use of droplet fission for a new application—the production of a large number of PEG microspheres—was demonstrated [35].

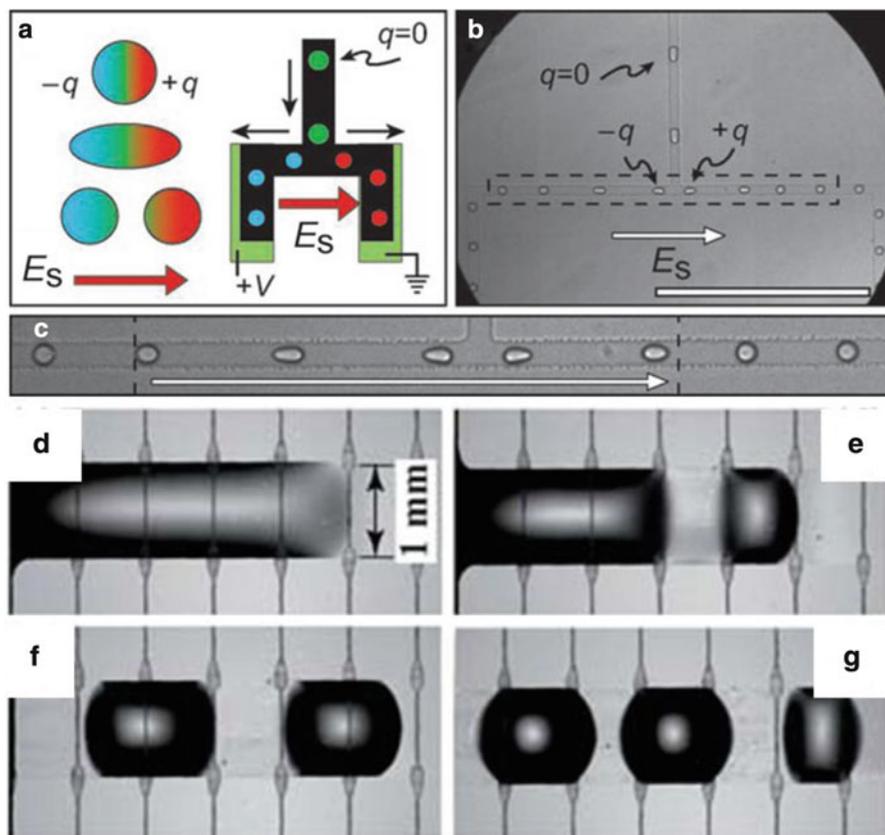
Another approach to combating the problem of asymmetric droplet splitting involved the use of syringe pumps to withdraw fluid evenly from multiple outlets. In a device that split a single droplet into 8, fluid was withdrawn from 7 of the 8 outlets at  $1/8$  the rate of the inlet flow. The eighth outlet of the device was left open to remove any excess fluid from the device. This technique minimized the pressure differences between the outlet channels and as a result, the device produced droplets with a size coefficient of variation of 9.38% [31].

Finally, a liquid sample can be split into nanoliter volume plugs by feeding the liquid into a main channel that splits into several smaller, daughter channels. The liquid fills each smaller channel until it reaches a valve. Mielenik et al. used a hydrophobic valve to arrest the flow of fluid into each daughter channel, while a waste channel was placed downstream of the daughter channels to drain excess fluid [36]. Once filled, each daughter channel contains 335 nL of fluid that can be metered out of each channel for further processing. The series of daughter channels effectively splits a single sample plug into eight smaller plugs. Using this technique, a single nucleic acid sample from a patient was split into ten smaller plugs, and each plug could be screened against a different reagent, allowing simultaneous screening for multiple viruses [37].

### ***2.3.2 Droplet Splitting Using Electrical Fields, Heat and Lasers***

While the aforementioned devices have the ability to split a droplet reliably into daughter droplets, the fission product volumes are constrained by the fixed geometry of these devices. Thus, several devices employing electrical fields, heat, and lasers have been developed to achieve more control over the droplet fission process and allow for dynamic adjustment of the daughter droplet volumes. In addition, such methods have the ability to “switch” droplets completely to one outlet or another, in lieu of executing droplet fission, so that only selected droplets may be divided if necessary. These additional functionalities come at the cost of a more complex device, but may be desirable or necessary, depending on the system’s application.

The throughput of droplet fission can be raised dramatically through the use of electric fields to split droplets. Link et al. used a device similar in geometry to the previously developed passive devices with bifurcating junctions, but added electrodes to charge and induce droplet splitting. Electrodes are placed under the two daughter channels after the bifurcation junction, and an electrical field



**Fig. 2.7** (a–c) Droplet splitting induced by an electric field (Reproduced from Link et al. [24], Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.). (d–g) Thermocapillary actuation, combined with chemical patterning of the device surface, was used to induce droplet fission through selective heating of the device surface (Reproduced from Darhuber et al. [41], by permission of The Royal Society of Chemistry, <http://dx.doi.org/10.1039/b921759b>)

is established between them. Uncharged droplets enter the bifurcation and the electrical field. The droplets polarize in the field and divide at the bifurcation (Fig. 2.7a–c). Simultaneously, opposite charges are induced on the two daughter droplets. Additionally, the authors noticed that at higher electrical fields, no droplet splitting occurred and the entire parent droplet was diverted to one of the daughter channels. Such a phenomenon could be used to remove erroneous droplets, or control the number or volume of daughter droplets produced in a bifurcating design. While this technique allows high throughput of up to 100,000 Hz, the use of electrical fields may preclude the use of this technique for some applications where reagents may be damaged by electrical fields [24].

Droplets may also be divided using a technique known as electrowetting-on-dielectric (EWOD). In this technique, electrical potentials are applied to specific points on a dielectric surface in order to induce movement of droplets by changing the wetting ability and contact angle of the fluid. Numerous groups have demonstrated the use of EWOD for droplet splitting [38, 39], however, the detailed mechanism and use of this technique is beyond the scope of this text.

One unique method for inducing droplet splitting involves the use of a laser. To use this technique, a microfluidic channel containing a post near the middle of the channel is used. In the absence of the laser, droplets split evenly around the post and form two daughter droplets of equal volume. However, when the laser is applied to one side of the post, the droplet is induced to split asymmetrically. Heat from the laser causes a local increase in the surface tension on the droplet, which prevents forward movement of the droplet past the laser [40]. The laser acts to block the droplet, and can affect the volume of the daughter droplets produced based on the length of time that the laser is applied in this position. As with the electrical fields, a droplet can be “switched” into a single channel instead of divided, by increasing the laser power used [16]. Although novel, this technique may not deliver as high a throughput as the technique in which electrical fields were used to split droplets, and may suffer from the same biocompatibility issue as well. However, the use of a laser provides even greater control over the volume of daughter droplets produced and may prove useful in devices where very precise manipulation of droplets is required.

Finally, microheaters integrated into microfluidic chips have been used to control droplet splitting and “switch” droplets to one of multiple downstream channels. In a technique similar to EWOD, Darhuber et al. used a technique called thermocapillary actuation, combined with chemical patterning of the surface of the device, to induce droplet splitting (Fig. 2.7d–g). The technique consists of stretching a fluid over a set of microheaters, activating the heaters sequentially to draw the fluid out, and then selectively turning off microheaters to induce splitting of the fluid [41]. Another group used an integrated microheater, positioned beneath one of the daughter channels (downstream of a bifurcation junction) to provide control over the volume of daughter droplets produced, as well as to allow switching of the droplet from one daughter channel to another. When the heater is turned on, a viscosity gradient is created and the viscosity of continuous phase in the heated daughter channel decreases. The fluidic resistance in the heated daughter channel is decreased owing to this decrease in viscosity. When a droplet reaches the bifurcation junction, a larger daughter droplet is produced in the heated daughter channel due to the difference in viscosity and interfacial tension between the two branches. By adjusting the temperature of the heater, different daughter droplet volumes can be produced. In addition, the use of an integrated microheater does not preclude the use of biological materials, since the heater works sufficiently for dividing droplets at 36°C. At slightly higher temperatures, the droplet does not split at the junction, but the entire droplet is carried or switched to the daughter branch containing the heater, providing a simple sorting mechanism [42].

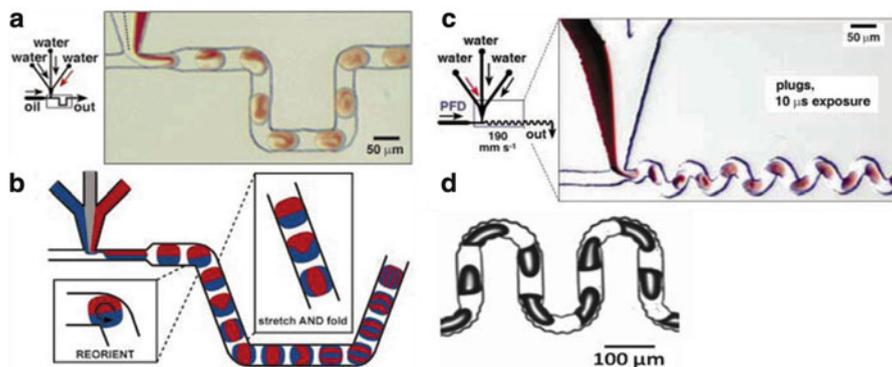
## 2.4 Droplet Mixing

One advantage to digital or droplet microfluidics over other types of microfluidic platforms is that droplets serve to contain the contents of a reaction. Owing to their small volumes (in the nanoliter or picoliter range) droplet contents mix more rapidly than the contents of a microtiter plate well, due to the decrease in diffusion distance alone. The advantages of rapid mixing in droplet microfluidics systems have motivated new methods for directly observing the kinetics of reactions with millisecond resolution [43], and will allow for higher throughput testing for many different types of applications.

When the droplet diameter is greater than the width of the channel and the droplet forms a plug, mixing within the droplet is enhanced by the contact of the droplet with the device wall. This contact induces the formation of a double recirculating flow pattern within the droplet, which increases the rate of mixing due to advection. This double recirculation flow has been observed optically [44] and measured, using particle image velocimetry [45]. Although the rate of mixing in such small volumes is relatively fast, high-throughput applications may demand even faster mixing. Several microfluidic devices to further enhance the mixing speed in droplets are discussed below.

Mixing in droplets is aided by recirculating flow inside the droplets, which is induced by shear forces from either the continuous phase fluid or the channel wall. However, the recirculation flow alone limits the ability of droplets to mix, since a double recirculating flow pattern does not allow for rapid movement of droplet contents across the centerline of the droplet. To overcome this limitation, winding channels have been implemented into microfluidic devices. As a droplet enters a winding channel, the shear forces experienced on either side of a droplet become uneven, and the droplet contents undergo a phenomenon known as “stretching and folding,” in which the recirculation patterns begin to cross the center of the droplet (Fig. 2.8a–c). The result of this folding action is that the distance over which diffusion must occur is shortened, thereby accelerating mixing of droplet contents. In addition, increasing the velocity of the droplets exaggerates the difference in shear force on either side of the droplet, and further increases the rate of mixing within the droplet [44, 45]. A mixing time of 2 ms was achieved using such a device. Another group studied the effect of the angle of the winding channels on droplet mixing, and concluded that a 45° winding channel provided faster mixing than either 90 or 135° bends [46]. The theory of mixing in droplets using these techniques is developed further elsewhere [47, 48].

To improve mixing further, several research groups have added bumps onto the edges of the winding channel design [49, 50]. This feature further enhances the asymmetry of shear forces on either side of the droplet as it traverses the winding channels, which aids mixing (Fig. 2.8d). In this design, one research group observed sticking of protein solution from droplets on the microchannel walls, but this problem was addressed by selecting a perfluorinated chemical as the continuous phase around the droplets [50]. Such a device was used for the detection of DNA in



**Fig. 2.8** (a–c) Mixing within droplets is enhanced by winding channels, which fold the fluid and decrease the diffusion distance within the droplet (a, b, Reprinted with permission from reference [47], Copyright 2003, American Institute of Physics. (c) Reprinted from reference [44], Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission). (d) Further agitation within droplets is induced by adding bumps to the edges of the winding channels (Reproduced from Fig. 2.3b of Hsieh et al. [49], with kind permission from Springer Science + Business Media BV)

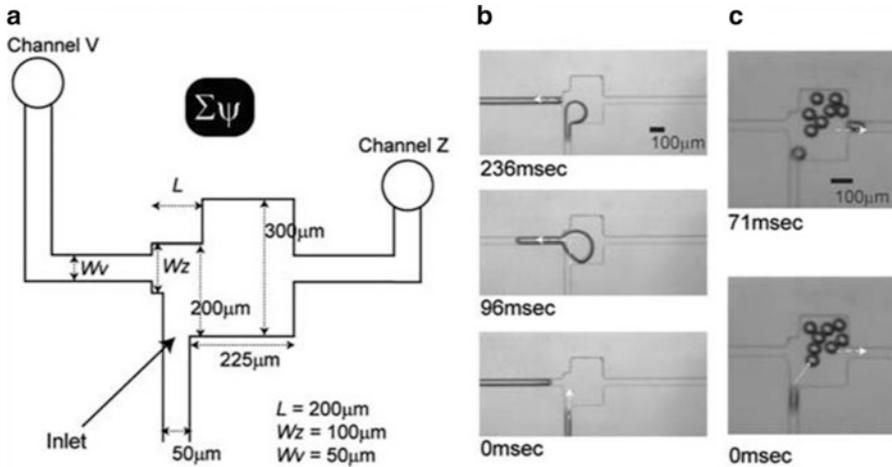
droplets using molecular beacons, and allowed for monitoring of the hybridization reaction in real time [49].

An alternative to the geometry-mediated methods of droplet mixing presented above is active mixing in droplet aided by lasers. By pulsing two lasers at high frequencies in a time-varying pattern, asymmetric mixing could be induced in droplets. The laser locally heats fluid inside the droplet and induces flow toward this heated spot. While novel, this technique may have limited applicability to microfluidic systems requiring high throughput, since droplets must be stopped in order for the mixing to occur. Hence, the maximum processing rate of droplets for mixing has been 1 per second. The advantage is that such a technique offers control over the duration and intensity of mixing [51].

## 2.5 Droplet Sorting

### 2.5.1 Passive (Hydrodynamic)

The simplest droplet sorting techniques require no detection or switching mechanisms, but instead rely on creative device geometry that allows the separation of droplets by size. By simply creating a bifurcating junction geometry in which the daughter channels had different widths, droplets were induced to sort into one of the channels (Fig. 2.9). For droplets within a certain size range, all of the droplets in a stream will sort into the daughter channel with a smaller width, since the shear is higher in this direction than the channel with a larger width [4]. A similar geometry



**Fig. 2.9** (a) Schematic of a microfluidic device for passive sorting of droplets by size. (b) *Large droplets* sort to the left due to higher shear on this side while (c) *smaller droplets* sort to the right due to a lower fluidic resistance in the shorter channel (Reproduced from Fig. 2.4a–c of Hsieh et al. [53], with kind permission from Springer Science + Business Media BV)

to exploit the streaming patterns of differently sized droplets was used to remove droplets much smaller than the desired size (termed “satellite droplets”) [52]. Building on the idea that large droplets could be diverted to a stream with higher shear, an asymmetrical bifurcating junction was created to separate large and small droplets based on two principles. The daughter channels from the bifurcating junction were designed with different widths as well as different channel lengths, to induce sorting based on both the shear at the bifurcating junction as well as the flow rate difference created by the difference in resistance due to channel length. While larger droplets were induced to sort into the narrower daughter channel, due to higher shear at that point, smaller droplets were sorted into the other channel [53]. Although simple to implement, the limitations of this technology are due in part to its fixed geometry. Only a certain range of droplet sizes and speeds are amenable to sorting using a given device with fixed dimensions. However, given a stable method of droplet generation, the size variation in produced droplets should not preclude the use of such a device to obtain monodispersed droplets after sorting.

One group used the principle of hydrodynamic sorting to sort droplets containing cells from those without cells. A hydrodynamic, flow focusing geometry was used to generate droplets from a stream containing cells in solution. Since the droplet generator was operated in the jetting regime, the droplets generated from the cell solution were normally small. However, when a cell approached the droplet generation junction, jetting was disrupted, and a larger than normal droplet was created. By applying uneven flow rates of continuous phase on either side of the cell solution phase, smaller droplets were directed toward the channel wall while larger droplets containing cells remained in the center of the channel. Then, the droplets containing cells were separated from those without cells downstream by dividing

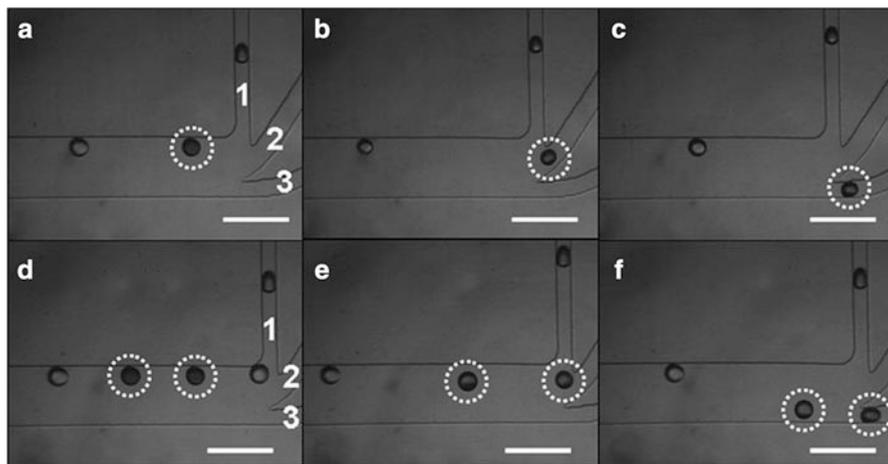
the main channel into several outlet channels. Such a device was able to process up to 160 cells per second, and the sorted droplet population was 99% pure. The device was later used to separate rare cells from whole blood, since T-lymphocytes, indicative of disease, are larger than red blood cells [54]. Hydrodynamic flow has also been combined with gravity-induced sedimentation in a device for the separation of polydispersed perfluorocarbon droplets into more monodispersed populations [55].

### **2.5.2 Magnetic**

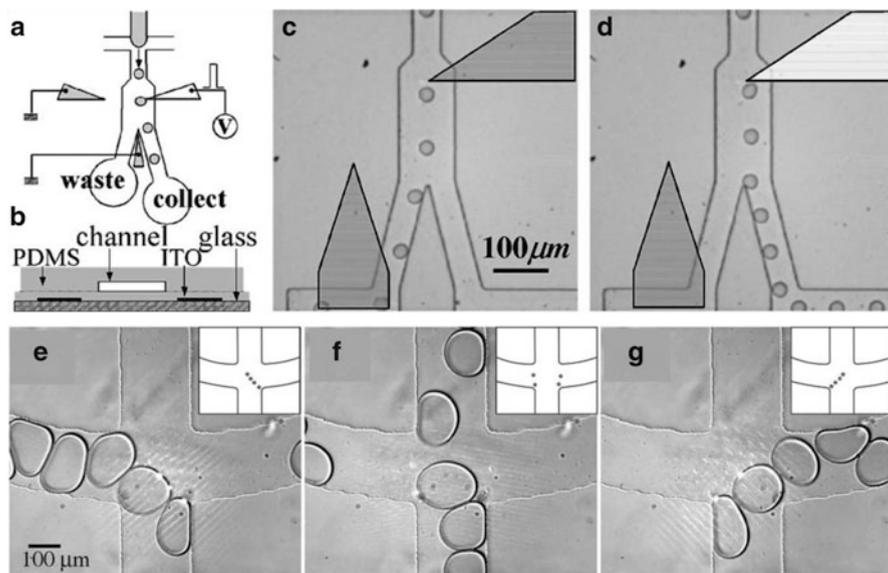
Just as magnetic particles have been used to tag and separate cell populations of interest, this technique can also be used to separate droplets containing magnetic particles from those which do not contain the particles. In 2004, a research group showed that such technology could be used to move aqueous droplets in air over a surface using a magnetic field. The aqueous droplets contained iron oxide nanoparticles, encased in silicon particles, which enabled control over the direction of the droplets [56]. Although efficient, the throughput of this approach was limited. Several years later, another group incorporated this concept into a microfluidic device to enable continuous sorting of droplets in this manner. Superparamagnetic magnetite nanoparticles were produced and incorporated into droplets, which could then be deflected into different channels by the targeted application of a perpendicular magnetic field (Fig. 2.10). To change the destination of the droplets and switch them to different outlets, the magnets were moved to different locations parallel to the main channel flow. This technique allowed separation of droplets at a rate of 10 per second. The use of such small magnetic particles ensures that they retain no magnetic “memory”, reducing the possibility of aggregation of the particles, and increasing their biocompatibility. Indeed, similar particles have been used safely in several types of biological assays [57].

### **2.5.3 Dielectrophoresis**

Just as dielectrophoresis has been used to facilitate the fission of droplets, it has also been used to sort droplets for further processing. The application of a nonuniform electric field exerts a force on droplets that can be used to direct droplets into one of several outlets of a device. In a device by Ahn et al., droplets flowed into the lower resistance outlet in the absence of DEP force. With the application of this force, however, the droplets were directed to a higher resistance outlet of the device (Fig. 2.11a–d). Sorting could occur at rates of up to 4,000 droplets per second using this approach [58]. For more precise manipulation of droplets, an array of  $128 \times 256$  individually addressable electrodes was designed and used to move droplets. Droplets could be moved over the surface of this array at speeds of up to



**Fig. 2.10** (a–c) Droplets loaded with magnetic nanoparticles are sorted into different outlet channels by positioning a magnet at different locations near the sorting region. (d–f) Consecutive droplets containing magnetic nanoparticles can be deflected in similar to the single droplets in panes (a–c) (Reproduced from Zhang et al. [57], by permission of The Royal Society of Chemistry, <http://dx.doi.org/10.1039/b906229g>)



**Fig. 2.11** (a–d) Droplets are sorted by dielectrophoretic force, imposed by application of potential across the two electrodes (Reprinted with permission from Ahn et al. [58], Copyright 2006, American Institute of Physics). (e–g) Patterned laser light is used to deflect droplets toward the desired outlet (Reprinted with permission from Cordero et al. [65], Copyright 2008, American Institute of Physics)

30  $\mu\text{m}$  per second by selectively activating electrodes. A lubrication layer of oil separate from the continuous phase surrounding the droplets facilitates movement of the droplets. This layer also prevents contamination between droplets during sorting. In addition, the application of DEP is made biocompatible by the application of an AC field, which is not as harmful to cells as DC fields [59, 60]. Several other devices employing dielectrophoresis with different electrode designs have been used for the separation of cells or beads [61–63].

In much the same manner as fluorescent cells can be sorted by a technique known as fluorescence-activated cell sorting (FACS), droplets containing fluorescent contents have been separated using dielectrophoresis. Such a device, termed a fluorescence-activated droplet sorter (FADS) detects passing fluorescent droplets and applies an AC field to direct fluorescent droplets to an alternate outlet channel. In the absence of the AC field, droplets are carried to the lower resistance outlet by default. A device by Baret et al. was able to achieve a sorting speed of up to 2,000 droplets per second, with a false sorting rate of 1 in 10,000 droplets, under optimal conditions for speed and accuracy, respectively. The FADS device was used to sort cells encapsulated in the droplets, and successfully distinguished between droplets with an active enzyme (which fluoresced) and those without the active enzyme (which did not fluoresce) [64].

### 2.5.4 *Optical*

For precise manipulation of droplets, focused laser light can also be used. In a device fabricated with a post structure in the middle of a microfluidic channel, a laser can be focused to one side of the post to prevent the droplet from passing the post on that side. After a droplet passes the post on the other side, hydrodynamic flow will direct the droplet into the corresponding branch of a bifurcation that follows the post structure in the channel. In this way, by positioning the laser on either side of the post, droplets can be sorted into one of two daughter channels. Although not addressed directly in the paper, the comparatively low throughput of the system as well as the biocompatibility of a laser could be issues [40]. To sort droplets into more than two daughter channels, a pattern of laser light spots has been used to direct droplets into one of three outlets (Fig. 2.11e–g). The sorting speed using a laser light pattern was between 30 and 60 droplets per second [65]. In a more complex approach, Kovac et al. enabled phenotype sorting of cells using an infrared laser—however, the technique could presumably be applied to sorting of cells encapsulated in droplets. Cells were first allowed to settle onto an array inside a microfluidic device, and cells of interest were identified manually by observation. Selected cells were lifted from the array using an infrared laser, into the flow through the device, and collected at the outlet. This approach avoids the manufacture and control issues associated with making an array of individually addressable electrodes. In addition, a low divergence laser beam was used in this method, which provided a large enough working distance to move the cells to the

desired area, while operating at a lower intensity than optical tweezers to avoid damaging the cells. One disadvantage to this approach is its low throughput—between 18 and 45 s were needed to sort a single cell [66]. Despite a lower throughput, these approaches offer a greater degree of precision in the sorting of droplets and may prove valuable for an application where the purity of the sorted population must be very high. Several other optical sorting approaches, including the use of optical tweezers and handles have been used for sorting particles or cells, and the technology could potentially be applied to droplet sorting as well [67].

### 2.5.5 *Other*

A handful of other techniques have been used to sort droplets, using electrical phenomena, fluid pressure induced by mechanical actuation, heat, surface acoustic waves (SAW), and others. An electric field imposed at a bifurcating junction has been shown in a device to induce sorting of droplets into one daughter channel or the other, depending on the direction in which the field is applied [24]. In another technique employing use of an electric field, selected droplets could be pushed into a separate stream by applying an electric field. Once selected, droplets were pushed into an aqueous stream flowing adjacent to the continuous phase flow, and the sorted aqueous droplets were absorbed into the continuous aqueous flow stream. Such a technique could be useful for downstream processing and enable droplets to be characterized using techniques that rely on analog, rather than digital flow, such as chromatography [27].

A unique approach to droplet sorting induced by mechanical actuation is the use of piezoelectric materials to produce a cross flow across the main channel of a microfluidic device containing droplets. To sort a droplet or train of droplets, a piezoelectric material is actuated, which depresses a PDMS membrane above a reservoir of continuous phase fluid. Depression of this membrane induces a flow of the continuous phase fluid in a channel perpendicular to the main channel flow. When streamlines from this side channel occupy at least 50% of the cross-sectional area of the main fluidic channel, droplets are diverted into a secondary outlet. Depending on the type of detector used to identify droplets for sorting, droplets could be sorted based on their volume or fluorescence [68]. Using similar device geometry, another group enabled droplet sorting by inducing electrokinetic crossflow in a device. In contrast to the device enabling crossflow using a piezoelectric actuator, fluid movement can be induced instantaneously upon activation of the electrodes which induce the electrokinetic flow. The disadvantage to this approach is that electrokinetic flow can only occur in a continuous phase where ions are present—thus the technique would not work well in a system where the droplets comprise the aqueous phase and the continuous phase is hydrophobic oil. Nevertheless, this sorting approach may be useful for some applications and was demonstrated successfully for the sorting of fluorescent beads from a stream of water [69], as well as cell sorting [70]. Franke et al. also demonstrated success in

droplet sorting using a piezoelectric actuator. In the absence of actuation, droplets in the main channel sort into the device outlet with lower resistance. For sorting, the piezoelectric material is actuated, which creates SAW that induces acoustic streaming to move droplets in the main channel. In this manner, droplets may be sorted into the higher resistance outlet, simply by actuating the piezoelectric material [71].

As mentioned earlier, passive sorting designs employ asymmetric bifurcation junction geometries to induce sorting based on flow rates and hydrodynamic resistance. Using a similar approach, Yap et al. induced droplet sorting by designing a device in which the fluidic resistance of the bifurcation junction daughter channels could be changed. A microheater, integrated into the microfluidic device, allowed switching of droplets into the higher resistance daughter channel following a bifurcation by heating the fluid in that channel. This heat reduced the hydrodynamic resistance in that daughter channel, which caused droplets to sort into the heated channel [42].

## 2.6 Conclusion

Although many varied and creative strategies have been devised for the manipulation of droplets—including the combination, separation, mixing, and sorting of these droplets—the potential for integration of these techniques into a complete processing device is what would eventually revolutionize the field and change the paradigm of how biological and chemical assays are carried out. Attempts to combine several droplet manipulation steps on chip have been successful for the execution of biological assays, but these techniques often depend on off-chip equipment for sample preparation or droplet storage [29]. The transition of microfluidic devices from a pursuit largely backed by academic labs to one endorsed and supported by industry will rely on the continued integration of multiple droplet processing steps in a single device. With the continued development of integrated microfluidic devices for droplet processing will come a reduction in processing time due to automation, a decrease in contamination potential by reducing manual handling steps, and a reduction of the cost of assays and reactions, due to a minimal consumption of all reagents involved.

## References

1. Bremond N, Thiam A, Bibette J (2008) Decompressing emulsion droplets favors coalescence. *Phys Rev Lett* 100:24501
2. Bibette J, Calderon F, Poulin P (1999) Emulsions: basic principles. *Rep Prog Phys* 62:969–1033
3. Hung L, Choi K, Tseng W, Tan Y, Shea K, Lee A (2006) Alternating droplet generation and controlled dynamic droplet fusion in microfluidic device for CdS nanoparticle synthesis. *Lab Chip* 6:174–178

4. Tan Y, Fisher J, Lee A, Cristini V, Lee A (2004) Design of microfluidic channel geometries for the control of droplet volume, chemical concentration, and sorting. *Lab Chip* 4:292–298
5. Liu K, Ding H, Chen Y, Zhao X (2007) Droplet-based synthetic method using microflow focusing and droplet fusion. *Microfluidics Nanofluidics* 3:239–243
6. Sivasamy J, Chim Y, Wong T, Nguyen N, Yobas L (2010) Reliable addition of reagents into microfluidic droplets. *Microfluid Nanofluidics* 8:409–416
7. Köhler J, Henkel T, Grodrian A, Kirner T, Roth M, Martin K, Metz J (2004) Digital reaction technology by micro segmented flow— components, concepts and applications. *Chem Eng J* 101:201–216
8. Tan Y, Ho Y, Lee A (2007) Droplet coalescence by geometrically mediated flow in microfluidic channels. *Microfluid Nanofluidics* 3:495–499
9. Um E, Park J (2009) A microfluidic abacus channel for controlling the addition of droplets. *Lab Chip* 9:207–212
10. Niu X, Gulati S, Edel J, deMello A (2008) Pillar-induced droplet merging in microfluidic circuits. *Lab Chip* 8:1837–1841
11. Simon MG, Lin R, Fisher JS, Lee AP (2012) A Laplace pressure based microfluidic trap for passive droplet trapping and controlled release. *Biomicrofluidics* 6(2):024103
12. Lin B, Su Y (2008) On-demand liquid-in-liquid droplet metering and fusion utilizing pneumatically actuated membrane valves. *J Micromech Microeng* 18:115005
13. Jin B, Kim Y, Lee Y, Yoo J (2010) Droplet merging in a straight microchannel using droplet size or viscosity difference. *J Micromech Microeng* 20:035003
14. Mazutis L, Baret J, Griffiths A (2009) A fast and efficient microfluidic system for highly selective one-to-one droplet fusion. *Lab Chip* 9:2665–2672
15. Fidalgo L, Abell C, Huck W (2007) Surface-induced droplet fusion in microfluidic devices. *Lab Chip* 7:984
16. Baroud C, Vincent M, Delville J (2007) An optical toolbox for total control of droplet microfluidics. *Lab Chip* 7:1029–1033
17. Shestopalov I, Tice J, Ismagilov R (2004) Multi-step synthesis of nanoparticles performed on millisecond time scale in a microfluidic droplet-based system. *Lab Chip* 4:316–321
18. Li L, Boedicker J, Ismagilov R (2007) Using a multijunction microfluidic device to inject substrate into an array of preformed plugs without cross-contamination: comparing theory and experiments. *Anal Chem* 79:2756–2761
19. Zagnoni M, Cooper J (2009) On-chip electrocoalescence of microdroplets as a function of voltage, frequency and droplet size. *Lab Chip* 9:2652–2658
20. Wang W, Yang C, Li C (2009) Efficient on-demand compound droplet formation: from microfluidics to microdroplets as miniaturized laboratories. *Small* 5:1149–1152
21. Tan W, Takeuchi S (2006) Timing controllable electrofusion device for aqueous droplet-based microreactors. *Lab Chip* 6:757–763
22. Priest C, Herminghaus S, Seemann R (2006) Controlled electrocoalescence in microfluidics: targeting a single lamella. *Appl Phys Lett* 89:134101
23. Wang W, Yang C, Li C (2009) On-demand microfluidic droplet trapping and fusion for on-chip static droplet assays. *Lab Chip* 9:1504–1506
24. Link D, Grasland Mongrain E, Duri A, Sarrazin F, Cheng Z, Cristobal G, Marquez M, Weitz D (2006) Electric control of droplets in microfluidic devices. *Angew Chem Int Ed Engl* 118:2618–2622
25. Ahn K, Agresti J, Chong H, Marquez M, Weitz D (2006) Electrocoalescence of drops synchronized by size-dependent flow in microfluidic channels. *Appl Phys Lett* 88:264105
26. Schwartz J, Vykoukal J, Gascoyne P (2004) Droplet-based chemistry on a programmable micro-chip. *Lab Chip* 4:11–17
27. Fidalgo L, Whyte G, Bratton D, Kaminski C, Abell C, Huck W (2008) From microdroplets to microfluidics: selective emulsion separation in microfluidic devices. *Angew Chem Int Ed Engl* 120:2072–2075

28. Tresset G, Takeuchi S (2004) A microfluidic device for electrofusion of biological vesicles. *Biomed Microdevices* 6:213–218
29. Mazutis L, Baret J, Treacy P, Skhiri Y, Araghi A, Ryckelynck M, Taly V, Griffiths A (2009) Multi-step microfluidic droplet processing: kinetic analysis of an in vitro translated enzyme. *Lab Chip* 9:2902–2908
30. Abate A, Hung T, Mary P, Agresti J, Weitz D (2010) High-throughput injection with microfluidics using picoinjectors. *Proc Natl Acad Sci U S A* 107:19163
31. Clausell-Tormos J, Griffiths A, Merten C (2010) An automated two-phase microfluidic system for kinetic analyses and the screening of compound libraries. *Lab Chip* 10:1302–1307
32. Hsieh A, Hori N, Massoudi R, Pan P, Sasaki H, Lin Y, Lee A (2009) Nonviral gene vector formation in monodispersed picolitre incubator for consistent gene delivery. *Lab Chip* 9:2638–2643
33. Link D, Anna S, Weitz D, Stone H (2004) Geometrically mediated breakup of drops in microfluidic devices. *Phys Rev Lett* 92:54503
34. Adamson D, Mustafi D, Zhang J, Zheng B, Ismagilov R (2006) Production of arrays of chemically distinct nanolitre plugs via repeated splitting in microfluidic devices. *Lab Chip* 6:1178–1186
35. Hsieh AT-H, Pan J-H, Pinasco PG, Fisher JS, Hung LH, Lee AP (2007) Polymer microsphere mass production using 128-channel digital fluidic chip. In: *The Eleventh international conference on miniaturized systems for chemistry and life sciences (uTAS 2007)*, Paris, France, pp. 346–348
36. Mielnik MM, Voitell J, Solli LA, Furuberg L (2007) Sample metering and parallel liquid plug actuation for multiple biochemical assays. In: *Eleventh international conference on miniaturized systems for chemistry and life sciences*, Paris, France, pp. 1513–1515
37. Furuberg L, Mielnik M, Gulliksen A, Solli L, Johansen I, Voitell J, Baier T, Riegger L, Karlsten F (2008) RNA amplification chip with parallel microchannels and droplet positioning using capillary valves. *Microsys Technol* 14:673–681
38. Cho S, Moon H, Kim C (2003) Creating, transporting, cutting, and merging liquid droplets by electrowetting-based actuation for digital microfluidic circuits. *J Microelectromech Syst* 12:70–80
39. Fan S, Hsieh T, Lin D (2009) General digital microfluidic platform manipulating dielectric and conductive droplets by dielectrophoresis and electrowetting. *Lab Chip* 9:1236–1242
40. Baroud C, Delville J, Gallaire F, Wunenburger R (2007) Thermocapillary valve for droplet production and sorting. *Phys Rev E Stat Nonlin Soft Matter Phys* 75:46302
41. Darhuber AA, Valentino JP, Troian SM (2010) Planar digital nanoliter dispensing system based on thermocapillary actuation. *Lab Chip* 10:1061–1071
42. Yap Y, Tan S, Nguyen N, Murshed S, Wong T, Yobas L (2009) Thermally mediated control of liquid microdroplets at a bifurcation. *J Phys D: Appl Phys* 42:065503
43. Song H, Ismagilov R (2003) Millisecond kinetics on a microfluidic chip using nanoliters of reagents. *J Am Chem Soc* 125:14613–14619
44. Song H, Tice J, Ismagilov R (2003) A microfluidic system for controlling reaction networks in time. *Angew Chem Int Ed* 42:768–772
45. Wang C, Nguyen N, Wong T (2007) Optical measurement of flow field and concentration field inside a moving nanoliter droplet. *Sensors Actuators A: Physical* 133:317–322
46. Sarrazin F, Prat L, Di Miceli N, Cristobal G, Link D, Weitz D (2007) Mixing characterization inside microdroplets engineered on a microcoalescer. *Chem Eng Sci* 62:1042–1048
47. Song H, Bringer M, Tice J, Gerdts C, Ismagilov R (2003) Experimental test of scaling of mixing by chaotic advection in droplets moving through microfluidic channels. *Appl Phys Lett* 83:4664–4666
48. Bringer M, Gerdts C, Song H, Tice J, Ismagilov R (2004) Microfluidic systems for chemical kinetics that rely on chaotic mixing in droplets. *Philos Transact A Math Phys Eng Sci* 362:1087
49. Hsieh A, Pan P, Lee A (2009) Rapid label-free DNA analysis in picoliter microfluidic droplets using FRET probes. *Microfluid Nanofluidics* 6:391–401

50. Liao A, Karnik R, Majumdar A, Cate J (2005) Mixing crowded biological solutions in milliseconds. *Anal Chem* 77:7618–7625
51. Cordero M, Rolfsnes H, Burnham D, Campbell P, McGloin D, Baroud C (2009) Mixing via thermocapillary generation of flow patterns inside a microfluidic drop. *New J Phys* 11:075033
52. Tan Y, Lee A (2005) Microfluidic separation of satellite droplets as the basis of a monodispersed micron and submicron emulsification system. *Lab Chip* 5:1178–1183
53. Tan Y, Ho Y, Lee A (2008) Microfluidic sorting of droplets by size. *Microfluid Nanofluidics* 4:343–348
54. Chabert M, Viovy J (2008) Microfluidic high-throughput encapsulation and hydrodynamic self-sorting of single cells. *Proc Natl Acad Sci* 105:3191
55. Huh D, Bahng J, Ling Y, Wei H, Kripfgans O, Fowlkes J, Grotberg J, Takayama S (2007) Gravity-driven microfluidic particle sorting device with hydrodynamic separation amplification. *Anal Chem* 79:1369–1376
56. Dorvee J, Derfus A, Bhatia S, Sailor M (2004) Manipulation of liquid droplets using amphiphilic, magnetic one-dimensional photonic crystal chaperones. *Nat Mater* 3:896–899
57. Zhang K, Liang Q, Ma S, Mu X, Hu P, Wang Y, Luo G (2009) On-chip manipulation of continuous picoliter-volume superparamagnetic droplets using a magnetic force. *Lab Chip* 9:2992–2999
58. Ahn K, Kerbage C, Hunt T, Westervelt R, Link D, Weitz D (2006) Dielectrophoretic manipulation of drops for high-speed microfluidic sorting devices. *Appl Phys Lett* 88:024104
59. Hunt T, Lee H, Westervelt R (2004) Addressable micropost array for the dielectrophoretic manipulation of particles in fluid. *Appl Phys Lett* 85:6421–6423
60. Hunt T, Issadore D, Westervelt R (2008) Integrated circuit/microfluidic chip to programmably trap and move cells and droplets with dielectrophoresis. *Lab Chip* 8:81–87
61. Choi S, Park J (2005) Microfluidic system for dielectrophoretic separation based on a trapezoidal electrode array. *Lab Chip* 5:1161–1167
62. Wang L, Flanagan L, Jeon N, Monuki E, Lee A (2007) Dielectrophoresis switching with vertical sidewall electrodes for microfluidic flow cytometry. *Lab Chip* 7:1114–1120
63. Li Y, Dalton C, Crabtree H, Nilsson G, Kaler K (2007) Continuous dielectrophoretic cell separation microfluidic device. *Lab Chip* 7:239–248
64. Baret J, Miller O, Taly V, Ryckelynck M, El-Harrak A, Frenz L, Rick C, Samuels M, Hutchison J, Agresti J (2009) Fluorescence-activated droplet sorting (FADS): efficient microfluidic cell sorting based on enzymatic activity. *Lab Chip* 9:1850–1858
65. Cordero M, Burnham D, Baroud C, McGloin D (2008) Thermocapillary manipulation of droplets using holographic beam shaping: Microfluidic pin ball. *Appl Phys Lett* 93:034107
66. Kovac J, Voldman J (2007) Intuitive, image-based cell sorting using optofluidic cell sorting. *Anal Chem* 79:9321–9330
67. Ozkan M, Wang M, Ozkan C, Flynn R, Esener S (2003) Optical manipulation of objects and biological cells in microfluidic devices. *Biomed Microdevices* 5:61–67
68. Shemesh J, Bransky A, Khoury M, Levenberg S (2010) Advanced microfluidic droplet manipulation based on piezoelectric actuation. *Biomed Microdevices* 12(5):907–914
69. Dittrich P, Schuille P (2003) An integrated microfluidic system for reaction, high-sensitivity detection, and sorting of fluorescent cells and particles. *Anal Chem* 75:5767–5774
70. Fu A, Chou H, Spence C, Arnold F, Quake S (2002) An integrated microfabricated cell sorter. *Anal Chem* 74:2451–2457
71. Franke T, Abate A, Weitz D, Wixforth A (2009) Surface acoustic wave (SAW) directed droplet flow in microfluidics for PDMS devices. *Lab Chip* 9:2625–2627