Manipulation of droplets in microfluidic systems

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Since the start of micro total analysis systems, manipulation of droplets in a microfluidic channel has been one of the most important branches of microfluidics. The scale of the droplet is remarkably small so that its mixing and reaction are rapid. With an array of channels and reliable programming, droplet microfluidics provides a high-throughput platform for applications in chemistry and biology. The droplet in a microfluidics system can be seen as an isolated reactor, with low consumption of samples and reagents, minimal dispersion and flexible control.

We review progress in manipulation of droplets in microfluidic systems and their applications. We also discuss future perspectives.

Keywords: Dispersion; Droplet; High-throughput platform; Manipulation; Microfluidics; Micro total analysis system; Mixing; Reactor; Reagent consumption; Sample consumption

1. Introduction

Microfluidics has developed rapidly since the start of micro total analysis systems (μTAS) [1–3]. It can perform typical laboratory operations with very low consumption of reagents and extremely short reaction times. Generally, in continuous-flow microfluidics, the flow is laminar at low Reynolds number. The properties of the microfluidics systems are closely related to their intrinsic characteristics:

1. consumption of sample and reagent is limited by the size of the micro-channel;
2. mixing in the channel is limited by diffusion; and,
3. dispersion, which is associated with pressure-driven laminar flow, leads to some problems (e.g., dilution and cross-contamination of the samples [4,5]).

These limitations can be overcome by confining samples and reagents in an immiscible carrier (i.e., droplets, which are spheres or plugs dispersing one fluid in another immiscible fluid and blocking all or part of the channel but not wetting the walls) [5]. The droplets can be formed, transported and sorted to realize various operations. When droplets containing different samples/reagents meet together, they can be merged and that merger immediately facilitates rapid chemical and biological reactions. The reaction or its products can be detected, categorized and reported using appropriate combinations of apparatus.

The kinetics of the reaction can be investigated in the droplet-microfluidics system [6]. An original droplet can also be split into small droplets for parallel manipulation or detection. Consequently, the manipulation of droplets with high precision and flexibility becomes a central issue, at which extensive investigations have been directed. Five approaches to manipulation of droplets can be found in the literature based on the sources of the force involved: hydrodynamic stress; electrohydrodynamics; thermocapillary; magnetism and acoustics. In the process of manipulating droplets, these forces have their own characteristics, in terms of formation, fusion, fission, mixing, sorting and transport of droplets.

Practically, the continuous phase should be able to wet the channel surfaces in order to keep the droplet stable and the wetting of the discontinuous phase should be different from that of the continuous phase. As an example, aqueous droplets in oil as the continuous phase need the micro-channel to have a hydrophobic interface. However, the oil droplets in the water phase require the micro-channel to have a hydrophilic interface. In this respect, the materials used for fabricating microchips are very important for forming and manipulating droplets. Some commonly used materials include polydimethylsiloxane (PDMS) [7–10], polymethyl methacrylate (PMMA) [11,12], quartzose [11], silicon [13–15]...
and glass [16]. Generally, much more interest is focused on PDMS because of its low cost, ease of fabrication, low Young’s modulus and transparency [7,8]. However, the presence of organic reagents has limited the application of PDMS due to it swelling and deforming.

So far, various surface-modification protocols have provided appropriate surface properties for the PDMS channel (i.e. hydrophobicity and hydrophilicity) [16]. When aqueous droplets are needed, the micro-channel surface can be modified to be hydrophobic by coating it with a thin layer of hydrophobic octadecyltrichlorosilane [17] or tridecafluorocholorosilane [18].

Surfactants have also been introduced into one of the phases to keep the droplets stable by reducing the surface tension between continuous and discrete phases. In that case, the surfactant molecules collected at the two-phase interface tend to form metastable colloids with their hydrophilic heads in the water phase and hydrophobic tails in the oil phase [11,14,19,20].

In practice, the presence of surfactant is unexpected when mixing droplets. In these circumstances, surfactant should be avoided in both oil and water phases.

2. Hydrodynamic manipulation

Hydrodynamic stress, generated by external mechanical force for driving two immiscible fluids, is a common method for manipulating droplets [19,21,22]. In this mode, the external power and the channel geometry are among the most important parameters.

The manipulation of droplets is realized by interaction of motive fluids and rigid geometries. In general, the forces include shear force, interfacial tension between the two immiscible phases [19] and high resistance to continuous fluid flow [21]. For different channel geometric designs, the mechanism of droplet formation differs. Although the mechanisms of droplet formation are still under investigation, the size of the droplet is influenced by the two-phase flow-rates and channel geometries [23].

As for the formation of droplets, hydrodynamic stress has a number of advantages (e.g., rapidity ($10^4$/s) [17], stability (no evaporation over 6 months) [24], uniformity [25], controllability of the amount of reagent in each droplet [26], and low cost of chip fabrication [27]). This method has been used to generate a wide range of droplets, including aqueous droplets in oil [19], viscous aqueous droplets in oil [28], oil droplets in water [11,15], and even aqueous droplets in ionic liquid [29]. Based on the geometry of the micro-channels, two typical modes are widely used for forming droplets (i.e., T-junction and flow-focusing). The two-phase liquids flow perpendicularly in the former mode and co-flow in the latter mode, with asymmetric and symmetric forces, respectively.

Considering that the flows in both modes are controlled by the geometry of the channel, the flexibility in droplet reconfiguration is limited. However, this approach can generate droplets with high efficiency, and is widely employed, so we devote more attention to it.

2.1. T-junction

Thorsen [19] was the first to use the T-junction for forming droplets in a microfluidics system by Thorsen. In this chip, the discontinuous phase in the branch channel is introduced perpendicularly into the continuous phase in the main channel. The competition between shear force and surface tension dominates the formation of droplets. Under the upstream hydrodynamic stress in the branch channel, the dispersed phase is forced to enter the main channel. Afterwards, the dispersed phase is subject to the shear stress of the flow of the continuous phase. Eventually, with assistance from surface tension, the dispersed phase breaks from the stream to form a droplet (Fig. 1).

The size of droplets depends on the flow rates of the two immiscible phases, the viscosity [28], the interfacial tension, and the geometrical dimensions of the device [21]. Oil and water flow rates particularly dominate the different spatial distributions of the two phases. [11] Change of the flow rates and their ratio will lead to different states of water phase (e.g., laminar flow, plugs, cobbles and drops).

![Figure 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph (from [19], courtesy of the American Physical Society).](http://www.elsevier.com/locate/trac)
On the basis of the single T-junction configuration, some improvements have produced highly uniform, monodisperse and stable droplets. A sudden increase in channel height downstream in the micro-channel facilitates the break up of droplets and formation of highly monodisperse droplets with coefficient of variance less than 1.5% [25]. A parallel microfluidic system with two T-junctions placed in parallel on the same chip produces emulsions with improved throughput [10].

As illustrated in Fig. 2, droplets have also been formed alternately in pairs at two liquid inlets [22,26]. If the first droplet is used for reaction, the other containing defined contents can indicate the composition of the first [26].

A two-T-junction structure in the channel forms monodisperse double emulsions [16], and the number of droplets can be readily controlled by adjusting the relationship between the break-up rates at the two junctions (Fig. 3).

2.2. Formation of droplets by focusing flow

The integration of a flow-focusing configuration into a micro-channel is frequently used to form droplets. Generally, there are three channels on the chip that converge into a main channel via an orifice [20]. The dispersed phase is contained in the middle channel, while the two side-channels contain the continuous phase. The two phases co-flow (Fig. 4a). The two phases flow through the small orifice and the dispersed phase is subjected to the forces generated by the continuous phase (e.g., pressure and viscous stresses, which are symmetrical compared to the forces in the T-junction). In such a case, it is easy for the dispersed phase to become narrow and break into droplets. The droplet size is usually related to the flow rates of the two phases and the flow-rate ratios [23]. Another factor affecting droplet size is the cross-section of the micro-channel, which can influence droplet formation at high flow rates. In a micro-channel with rectangular cross section, the water stream was flanked by two oil streams through a narrow rectangular gap [20]. When rapid, stable formation of droplets is needed, it is necessary to increase the flow rates, so as to ensure that the central flow is axisymmetrically confined, and that increases the probability of chip leakage (Fig. 4a).

A circular constriction broke through this limit [17] by expanding both channel height and channel width, as shown in Fig. 4 (b and c), where a concentrated, ring-shaped pressure was exerted on the dispersed phase, which made droplet break up more spontaneous and precise with a rapid speed (i.e. exceeding 10^4/s) for water-in-oil droplets and reaching 10^3/s for oil-in-water droplets [17].

In order to achieve high throughput and more complex operations, multiple flow focusing could be used in parallel [30] and simultaneously form droplets with different dimensions.
However, the droplets formed in the flow-focusing device are usually followed by formation of some satellite droplets [18], which reduce droplet monodispersity, so it is necessary to provide approaches for filtering or eliminating the satellite droplets or improving their formation [31]. Fig. 5 illustrates that, by adding two pneumatic air chambers to the sides of a flow-focusing micro-channel, the sheath flows can be locally accelerated to adjust the size and the orientation of the droplet, when activating the moving-wall structures by injecting air [32].

2.3. Other manipulations
In droplet microfluidics, not only is droplet formation important, but other manipulations are too (e.g., fusion, fission, mixing and sorting). As independent reactors, the droplets, comprising chemical or biological sample, reagent or micro-particles, need to be driven to realize reactions (e.g., chemical reaction, biological response or synthesis). These require further techniques to manipulate droplets. Hydrodynamic stress is a simple and effective method to accomplish manipulation(s) of droplets, also related to the geometry of the micro-channel.

2.3.1. Fission. Droplet fission has been a very important issue in droplet-based microfluidics systems. It generally includes the following aspects: reducing the droplet volume; controlling the concentration of chemicals inside the droplets [33]; and, producing arrays of droplets for high-throughput (Fig. 6) [34].

Fission of droplets can be carried out controllably by hydrodynamic stress and a bifurcating junction. When the two-phase fluids in the main channel flow toward a bifurcating junction, the droplet is affected by the pressure and the shear strain arising from the flow. As long as the forces surpass the interface tension, fission occurs, so decreasing the inlet width of the main channel or constricting the channels at the branching points can increase the forces on a droplet and lead to its split [35]. The relative sizes of daughter droplets depend on the symmetry of the flow. If the flow is fully symmetric, equal forces will be exerted onto the two halves of the mother droplet, resulting in the creation of two equal-sized daughter droplets [34,36]. For asymmetric flow, the forces on the two halves of the mother droplet are proportional to the droplet surface area exposed to those stream lines, and the mother droplet tends to break up into two unequal daughter droplets. The volume of the daughter droplet therefore depends not only on channel resistances but also the volume of the mother droplet [33]. Under asymmetric flow, daughter droplets with different concentrations would be produced from a primary droplet if the concentration gradient of mother droplet was retained until fission occurred [33].

2.3.2. Fusion. Chemical and biological analysis commonly needs coalescence of different liquids (e.g., samples and reagents) to complete the reactions. In droplet microfluidics, this approach is replaced by fusion of droplets. Introducing some substances into a droplet and merging two droplets with different contents are both indispensable. In practice, merging droplets should at least fulfill the following pre-requisites: touching each other; and, overcoming the stabilizing forces caused by surface tension and lubrication.

So far, extensive investigations have been directed to ensure contact of two adjacent droplets. A number of novel configurations have been constructed in the micro-channel to make the droplets meet each other (e.g., small and big droplets moving at two different velocities will coalesce until they enter a wide main channel, due to the difference of flow pressures in the two inlet channels with different dimensions [35]). A recent flow-rectifying design facilitates simultaneous fusion of three or more droplets [27,33]. In addition, a tapered expansion in a micro-channel generates a velocity gradient...
which allows approach to the droplets, oil-film drainage between the droplets, and finally leading to the fusion of the droplets (Fig. 7).

To overcome the stabilizing forces related to surface tension and lubrication, fusion depends on the viscosity ratio of the internal and external fluids as well as the presence of surfactant at the interface. When the internal phase (i.e. the droplet) has a lower viscosity, the film between the immiscible fluids will be easy to drain and rupture, facilitating coalescence of the droplets [26]. However, by contrast, if the droplet has a higher viscosity, the interface is less mobile, and it is therefore more difficult for the two droplets to coalesce. In addition, presence of surfactant at the interface of two droplets also arrests coalescence [37,38].

2.3.3. Mixing. The degree of mixing for the reagents is a governing factor in the reaction. For rapid analysis, effective mixing is therefore most important. In continuous-flow microfluidic mode, laminar flow is associated with low flow rate and small cross-sectional dimension, so diffusion is the only way to mix fluids. Similarly, diffusion also makes a major contribution to mixing in droplets. First, mixing is affected by striation length (i.e. the length of fluid over which mixing occurs). A shorter striation length is obviously favorable for fast mixing. As a result, reducing the dimension of the droplets can significantly shorten the time of diffusion-controlled mixing. Furthermore, if the initial droplet has a proper distribution of aqueous reagents, mixing will be relatively optimal. However, the initial distribution of the contents in the droplet depends strongly on droplet formation [5].

As an alternative, chaotic advection facilitates rapid mixing of multiple reagents isolated in droplets in the sub-ms range [35,39,40]. Ismagilov’s group has made great efforts to mix droplets by hydrodynamic stress [5,28,35,40]. They found that the droplet should be large enough to be in contact with all four walls of a micro-channel to ensure chaotic advection, which is caused by periodic recirculating flow inside the droplets, resulting from the shearing interaction of the droplets with the micro-channel walls. When a droplet flows through a winding microfluidic channel, chaotic advection makes the liquid in the droplet mix fast (Fig. 8).

2.3.4. Sorting. Droplet sorting is necessary to distribute droplets into different downstream micro-channels for further use and to harvest monodisperse droplets for precise analysis. In this process, droplets are sorted by hydrodynamic stress mainly based on the geometry of the micro-channel. At an asymmetrical bifurcating junction, the flow is divided into unequal streams based on the flow rate and the width of each daughter channel. The droplet with the larger surface is affected by greater
hydrodynamic stress and enters the daughter channel at a higher flow rate [31,33], while the smaller droplets enter another daughter channel. However, sorting is not completely efficient for all droplets, so further investigations are required in this respect. Lee’s group collected satellite droplets with diameters of ~1 mm with a loop structure in one of the daughter channels [33]. Afterwards, the location of droplet formation was regulated to let the small droplets avoid the high-stress stream line, and monodispersed and bidispersed satellite droplets with μm and sub-μm sizes were collected simultaneously, as shown in Fig. 9 [31]. The even distribution of

![Figure 5](image-url)  
*Figure 5.* (a) SEM of PDMS micro-channel with air chambers. (b) and (c) Formation of droplets with tunable moving-wall structures: (b) extracting air from a chamber (c) injecting air into a chamber (from [32], with kind permission of Springer Science and Business Media).

![Figure 6](image-url)  
*Figure 6.* Splitting from a single-droplet array to 16 small-droplet arrays (from [34], with kind permission of the Royal Society of Chemistry).
droplets can be accomplished with a symmetrical bifurcating junction.

Generally, hydrodynamic resistances are unsteady and complex in the process of sorting into each branch channel, which is closely related to the number and the size of the droplets contained in the channel, so sorting is non-linear [41]. To solve this problem, a bypass can be introduced at the junction [42]. When a droplet enters branch 1, resistance in the branch increases. Simultaneously, the bypass, through which the droplet cannot pass, transfers the difference of resistances to branch 2. As a consequence, the flow rate in branch 2 increases, and the next droplet in the main channel enters branch 2 (Fig. 10). By doing so, an even distribution of droplets between the two outlets of a T-junction can be achieved.

For droplet systems manipulated by hydrodynamic stress, channel geometry is most important for controlling the forces that create, transport, sort, mix, split and fuse the droplets. For a design to be successful, the following factors should be carefully considered:
- droplet-solid contact angles;
- interfacial tension at the two-liquid interface;
- the stir induced by the droplet movement; and,
- the shear force generated by the carrier flow around the droplets.

This indicates a trend of future research in the field (i.e. from empirical design to rigorous study of the flow characteristics). There is therefore still plenty of room for improvement by theoretical investigations and direct calculations of flow [27].

3. Electrohydrodynamic manipulation

The electrohydrodynamic (EHD) force generated by an electric field is also widely used to accomplish the basic operations in a droplet-based microfluidics. At the μm scale, interfacial tension forces dominate the hydrodynamic behavior of the droplets, so EHD forces used in the manipulation of droplets must first overcome or change the interfacial tension. According to energy-transduction mechanisms [43], two typical EHD methods are available (i.e. electrowetting (EW) [4,44] and dielectrophoresis (DEP) [45]). Generally, for conductive liquids, the EW force might dominate the formation of droplets. However, for dielectric liquids, both DEP and EW might
contribute, and the DEP force may dominate the process [46].

3.1. Electrowetting
Electrowetting for the formation of droplets was first reported by Washizu [4]. When a water droplet is in contact with a solid electrode coated with a hydrophobic film, a wetting force may arise upon application of an electric field. This wetting force can reduce the contact angle, and difference in wetting force arising from an electric field can make a droplet move [43,44]. Fig. 11 showed that electrolysis might occur when a high voltage is applied. By naming the voltage of electrolysis as the critical voltage, an insulating layer can be inserted between the droplet and the electrode so that the critical voltage is raised and electrolysis is avoided, acquiring a stronger wetting force. This so-called electrowetting on dielectric (EWOD) [47] has become one of the most promising ways to manipulate droplets in microfluidic systems.

Generally, EWOD devices are fabricated on two-plane devices. The droplet is sandwiched between the two electrode planes and surrounded by gas, silicone oil or another immiscible liquid. The channel is wetted by the fluid when activating the electrodes and the fluid begins to form a short liquid finger between the electrodes.

Figure 9. Channel design for controlling the dynamic separation of satellite droplets. It comprises a droplet-generation region and a separation region. The separation region separates the satellite droplets according to their position across the width of the channel. (Bottom) Parent droplets are collected into the mid-collecting zone while the satellite droplets can be switched into either the top or bottom collecting zone (from [31], by permission of the Royal Society of Chemistry).
When the electrodes are switched off, the surface reverts back to hydrophobic, causing the finger to break off from the reservoir or mother droplet, and to form a new droplet or daughter droplet [43] (Fig. 12).

By sequentially applying voltages underneath control electrodes, the droplet can be moved in the direction of the activated electrode. This is the typical process for transporting droplets. When adjusting the procedure to activate the electrodes, the daughter droplets may be moved towards each other and finally merge.

The size of the droplet depends on the strength and the frequency of the electric field, as well as the width of the channel opening. For example, higher frequencies produce smaller droplets. The concentration of daughter droplets can be controlled by combining fission and other techniques (e.g., electrophoresis [48]). Three modes of mixing based on EWOD are available:

1. based on diffusion inside an immovable droplet;
2. by oscillation while the droplet is moving; and,
3. during the process of a droplet splitting into two droplets and then remerging [49–51].

The key to accurate accomplishment of each process of manipulation is to design the size and the configuration of the array of independently-addressable control electrodes [52,53]. This needs complex, refined micro-fabrication techniques. In addition, EWOD may cause surface contamination in real world biological analysis, because translational electrowetting forces depend closely on the difference of wetting between leading and trailing edges of droplets while the surface is less likely to be even and clean.

3.2. Dielectrophoresis

DEP is another method of manipulating droplets based on electrically neutral but polarizable fluid [46], a non-uniform electric field and the movement of droplets to the regions of maximum electric field intensity [3,53–55].

Generally, a DEP system contains coplanar electrodes (i.e. smooth and insulating substrate), covered by a thin dielectric layer, and the fluid involved has higher dielectric permittivity than its surrounding fluid. When the fluid remains on the hydrophobic substrate, it can be transported and divided into hemispherical nanodroplets by short application of voltage and appropriate change in electrode connections [54,55] (Fig. 13).

The droplets can be sorted into the collection stream by applying an electric field on the electrodes to generate a DEP force [56].

Figure 10. Schematic (left) and snapshot (right) of two junctions in the same device fed by identical means with outlet branches of equal lengths connected to atmospheric pressure. Dashed lines have been added to the photograph for clarity. The left junction has a bypass, and shows a perfectly alternating distribution of droplets between its two outlets. The right junction has no bypass, and the droplets are dispatched through a random-like alternation of long trains (from [42], by permission of the American Institute of Physics).
In practice, the dielectric permittivity of the droplet is higher than that of the surrounding fluid, so joule heating is inevitable. The increase in temperature can be controlled effectively with a proper electrode design and an operational mode where voltage is applied for very short time [55]. When using a DEP technique to manipulate droplets in a direct current or low-frequency alternating current field, fast formation (0.1 s) [45] and sorting (1.6 kHz) of droplets can be achieved [56].

3.3. Combination of electric control and other methods
Sometimes, the manipulation of droplets by a single method is monotonous and insufficient for flexible applications. The combination of an electric field with other methods (e.g., hydrodynamic stress) can also perform droplet manipulations comfortably. By combining hydrodynamic stress and electric field, fusion can be realized in a simple geometric device [57–60]. As illustrated in Fig. 14a, the adjacent droplets in two arrays were merged by applying an electric field on vapor-deposited-gold electrodes on the cover plate of a micro-channel [57]. A double-T-junction can form two series of droplets with same or different compositions [58], and a non-uniform electric field can be generated by applying a DC voltage across the electrodes embedded under the junction. Consequently, the adjacent droplets will merge (Fig. 14b). Fig. 14c shows that, by applying an increasing electric field, droplets are attracted closer, thus initiating their fusion [60].

A system incorporating an electric field and flow-focusing geometry can realize the functions of creating, recombining, splitting and sorting droplets [61]. By applying high voltage to the aqueous stream and charging the oil-water interface, an electrically-addressable emulsification system was created [61]. To generate droplets, the electric field assisted to reduce the dependence of the droplet size on the flow rate and the channel dimensions (Figs. 15 and 16). A pre-requisite for such a system is that the discontinuous phase should be conductive and charged.

From the above discussion, we can see that, to manipulate droplets, self-contained EHD requires no moving parts or fixed channels, consumes little power, and imposes minimal constraints upon the fluid involved. In addition, EHD is flexible and suitable for manipulating single droplets or a small number of droplets. However, it needs fabrication of electrodes or electrode arrays on the micro scale.

Considering that hydrodynamic stress can form droplets, while EHD is able to reconfigure the droplets, both approaches deserve further investigation and more protocols are required to combine them.

4. Droplet manipulation by thermocapillary, magnetic actuation and acoustic radiation
Except for the approaches mentioned above, some other protocols for manipulating droplets have also been exploited. Because the micro-scale droplet is mainly controlled by surface tension, successful manipulation of droplets will be closely related to overcoming the surface tension. At present, the methods based on thermocapillary [62,63], magnetism [64–66] and acoustics [67] are successfully used to manipulate droplets in microfluidic systems, as discussed briefly in the following sections.

4.1. Thermocapillary
Thermocapillary is a phenomenon that occurs with the change of the surface tension at a two-phase interface due to temperature variation. It can be cited as a mechanism for driving droplets immersed in a second immiscible phase. Interfacial tension usually decreases with increase in temperature [68,69]. When the droplet lies in a temperature gradient, the tension exerts a tangential surface force that pulls liquid toward the cold spot [70–73]. In this respect, droplets can be driven by the thermocapillary effect, depending on the means of heating. By using integrated microheater arrays for the formation of temperature gradients in combination with partial wetting surfaces, droplet transport can be facilitated without volume loss and cross-contamination.
between individual droplets is virtually eliminated [72].
With a focused laser for providing local heating of a
liquid interface, the thermocapillary force can prevent
the advance of a droplet in a micro-channel [73,74]. By
combining with a T-junction as a contactless optical
microfluidic valve, the laser can also be used to control
droplet formation, sorting, fusion and division (Fig. 17).

Droplets of fL volume generated in a microfluidic
system can be manipulated and transported by using
optical vortex traps [75]. Shah integrated electrowetting-on-dielectric (EWOD) and optoelectronic tweezers (OETs)
to isolate and to analyze cells [76]. OETs manipulated
individual particles (e.g., cell) to accumulate in one part
of a droplet, and then EWOD split the droplet into a
concentrated daughter droplet and a diluted one. Based
on these two techniques, a series of droplet manipula-
tions can be accomplished.

In addition, droplets can be formed simply by light
scattering-induced flow. Casner’s group [77,78] used a
laser to shine onto a soft, near-critical liquid-liquid
interface. When the beam with modest powers traveled
from the phase with a higher refractive index to the
phase with a lower refractive index, a jet of the upper-
layer liquid formed along the beam axis and the droplets
regularly formed at the end of the jet. We predict that
this method would also be effective in forming droplets as
long as suitable droplet manipulations with other func-
tions are available in the system.

4.2. Magnetic actuation
Magnetic actuation is unique because it is independent
of surface charge, pH and ionic strength, so it is com-
patible with a wide range of substrate materials and bio-
chemical processes. It needs only a simple device to in-
clude a reservoir and a magnet. The process involves the
formation of droplets containing magnetic beads and the
droplets are moved by the draw force of the magnetic
beads actuated by an external magnet. The external
permanent magnet or electromagnet remotely controls the superparamagnetic particles inside the droplet. The actuation is affected by particle type, droplet size, surrounding oil layer, surface tension and viscosity [79]. The quantity of superparamagnetic beads in each droplet also decides whether the motion of droplet is successful or not. It is worth mentioning that, for aqueous droplets, the surface of magnetic beads should be hydrophilic, so that the force due to their interaction with water can force the droplets to move. In this respect, silica is usually coated on magnetic beads to form hydrophilic surfaces. In addition, magnetic beads play important roles in polymerase chain reaction (PCR) [64,80–82], sample isolation and preconcentration [65,83,84] and immunoassay [66,85].

Electrostatic forces and magnetic forces have been used to anchor and to drive droplets on hydrophobic substrate [65]. This method could be applied to biochemical processes, (e.g., dilution, washing, extraction and purification). Hence, magnetic actuation has capability in droplet-based microfluidics, and the combination of magnetic force with other forces might result in more effective approaches to driving droplets.

4.3. Acoustic radiation

Acoustic radiation from a surface wave leads to internal streaming in the fluid and eventually to form small droplets along predetermined trajectories. Chemical modification of the planar surface of the piezoelectric chip is employed to modulate wetting properties of the surface and define a fluidic network. It does not need a micro-channel or a micro-valve, but operates in an open system [67].

Surface acoustic waves (SAWs) on a piezoelectric substrate can produce acoustic radiation in the fluid. This stress is basically the origin of SAW-mediated internal streaming in the fluid and small droplet formation. When a droplet passed through the modified surface, it was dispensed to remain small on the surface or merged with the original droplet at the location. The combination of acoustic radiation with a sensor or a heater might bring more attractive application of the droplets [67].

5. Applications

Due to the distinct advantages of minimized sample and reagent consumption, improvement in mixing efficiency and flexibility in driving droplets containing different samples and reagents, droplets in microfluidic systems have vast potential in high-throughput sample pretreatment, chemical analysis, micro-particle synthesis and biological assays. The droplet-microfluidic system shows a sharp increase in reaction rate due to the high ratio of surface to volume and high local concentration of reactants. With the advance in related techniques, the application of droplets in microfluidic systems will play a more important role in microfluidics in not only chemistry but also biology. Here, we briefly summarize progress in these fields. Other recent reviews [3,86,87] contain further surveys regarding applications of droplets.

5.1. Sample pretreatment and chemical analysis

Based on the numerous advantages of droplets, many routine sample-pretreatment processes in chemical analysis were devised in droplet microfluidics (e.g., extraction and preconcentration [29,67,88–90]).

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**Figure 13.** Dielectrophoresis with planar electrode configurations for wall-less flow structures. (a) Cross-section of original microelectrode structure used with insulating transformer oil. The liquid forms a semicircular profile centered on the gap. (b) Side view of dielectric height-of-rise experiment showing a finger of liquid extending upward in the gap between the coplanar electrodes (from [54], by permission of the American Institute of Physics).
Chen reported a microfluidic chip-based liquid-liquid extraction and preconcentration system using a sub-nL-droplet-trapping technique, and achieved high enrichment factors (>1000), with sample consumption of only a few μL [88].

A water-immiscible ionic liquid (IL) was employed as continuous phase for wrapping water droplets on flow-focusing microfluidic chips [29]. This system also achieved high efficiency in water/IL extraction. In the chemical analysis of individual sub-cellular organelles, the number of molecules to be analyzed in a droplet is very limited, and therefore preconcentration of the analyte is highly desired to improve the detection capability. In this respect, the aqueous droplets can be dissolved into the surrounding organic phase [91]. As the droplet shrinks the concentration of the analyte in the droplet will be increased. The screening of organic reactions can also be carried out in the droplet-based microfluidic system. The discrete droplets can be used as microreactors for synthetic reactions of selective deacetylation of ouabain hexaacetate [92]. These investigations show that the droplet-based microfluidics has vast potential in investigation and optimization of reactions with precious reactants, especially for time-consuming reactions.

5.2. Synthesis of micro-particles

In general, the synthesis of micro-particulate materials is not easy to control. However, droplet microfluidics can accomplish syntheses of monodisperse polymers and particles with low consumption, good quality and high speed.

A multiple-step synthesis of CdS and CdS/CdSe core-shell nanoparticles on millisecond time scale was demonstrated in a PDMS device, with benefit of the winding channels in which the reagents in droplets were rapidly mixed [93].

High-temperature synthesis of CdSe nanocrystals in nL-volume droplets was also presented [22,94], and a versatile strategy was reported for producing monodisperse solid particles with sizes of 20–1000 μm [97].

Similarly, monodisperse polymers (e.g., polydivinylbenzene macrobeads 100 μm in diameter) were prepared by using drop breaking and polymerization [95].

Continuous droplet formation and in situ photopolymerization in microfluidic devices generate uniform-sized monodisperse poly(ethylene glycol) (PEG) microspheres with a coefficient of variation (CV) of <1.8% [96].

In a droplet-based microfluidic system, pre-packed droplets solidified in situ polymerization from a liquid monomer generating droplets. By controlling the volume of the individual droplets and the cross-sectional area of the micro-channel, both shape and size of the solidified beads could be manipulated, resulting in beads of spheres, disks, ellipsoids and rods [97].

When encapsulating anticancer drug tamoxifen, Fe₃O₄ nanoparticles and CdTe quantum dots into
size-controlled polycaprolactone droplets in a cross-junction micro-channel, magnetic targeting, fluorescence imaging and controlled drug-release properties could be integrated into a single drug-delivery system [98].

5.3. Biological

For most biological analytical systems, generally only a very small amount of biological analyte is available, so low consumption of sample becomes a key issue, and the miniature analytical system has become a most popular platform for biological investigations [67], where the speed of reaction increases due to decreasing sample and reagent consumption. Recent developments have indicated that droplet microfluidic systems have obvious priority in the field of biological analysis [99] (e.g., DNA analysis [67], cell analysis [100], enzymatic assay [100–103] and protein analysis [104]). In the following sub-sections, we address, a few important aspects of droplet microfluidic systems in biological applications.

5.3.1. Droplet PCR. PCR has been a very important, efficient method in DNA analysis. The combination of PCR with droplet microfluidics offers obvious advantages (e.g., minimized consumption of reagent and sample and significant time saving). When employing a magnetic bead-droplet-handling system for PCR, the droplet containing magnetic beads suspended in oil phase was
moved by a magnet, and the PCR mixture in the droplet was pulled to pass specified temperature regions, where amplification was achieved [105]. By developing a temperature gradient along the route of the moving droplet with parallel channels to realize multi-droplet PCR, rapid PCR amplification could be achieved within 3.6 min for 40 cycles [64].

The strategy of multi-droplet PCR can also be performed by magnetic actuation in the multi-round channels [82]. PCR amplification could be realized with a channel-less device, based on a magnetic droplet [80]. The THP-1 cell from blood droplet was first purified and then merged with the cell-lysis/PCR mixture droplet. After lysis of the cell, PCR was started so that high-speed, high-quality product was obtained.

Furthermore, high-throughput isothermal amplification of a single DNA molecule in a 2-µL droplet has been demonstrated successfully [106].

5.3.2. Encapsulation of cells. A specific cell can be encapsulated in a droplet for single-cell analysis [107,108], as the droplet provides a reduction in volume and an enclosing environment in cell analysis with low consumption of reagent and sample, and high throughput.

By using optical trapping and a droplet-based microfluidic system, a single target cell or sub-cellular structure could be encapsulated into a µL or nL aqueous droplet surrounded by an immiscible phase [100]. Thereafter, rapid laser photolysis of the cell and assay of the activity of an enzyme (i.e. intracellular enzyme β-galactosidase) could be carried out.

A T-junction is effective for encapsulating single cells in droplets [109]. The cell-containing aqueous droplets could be frozen to confirm the viability of the cell. Moreover, a cross-junction can encapsulate not only mammalian cells but also multi-cellular organisms in droplets and keep them proliferate for several days [110].

5.3.3. Enzymatic assay. Enzymatic assay has been one of the most important issues in biological investigations, for which droplet-based microfluidic systems are powerful, as kinetic measurement of enzymatic reactions in ms could readily be realized based on rapid mixing and multi-injections [6]. Such a microdevice, combined with multiple junctions, facilitates injection of substrate into the droplets, which minimize the probability of cross-contamination and achieve multi-step reactions. Both evaluation of enzymatic activity of thrombin and determination of the coagulation time of human-blood plasma were realized in such a droplet-based microfluidic system [102,103].

Another interesting work in this field is enzymatic reaction by fusion of µL droplets containing β-galactosidase and fluorescein di-β-D-galactopyranoside [60].

5.3.4. Protein crystallization. Crystallization is a bottleneck in determining tertiary protein structures from sequence data [104]. In protein crystallization, screening the concentrations of protein and reagents (precipitants, buffers and additives) is usually time consuming and tedious. In some circumstances, it consumes a large amount of rare or precious reagents and samples, so that there is great demand to minimize consumption of proteins during the screening process because some of the proteins are available in very small quantities only. With this in mind, some investigations have been directed at protein crystallization in microfluidic droplets [24,35,111–115].

Screening hundreds of protein-crystallization conditions was carried out in a droplet microfluidic system at a rate of several trials per second under computer control by using <4 nL of protein solution for each crystallization trial. The crystallization trials were set up inside 7.5-nL aqueous droplets. The quality of the crystals was evaluated by direct on-chip X-ray diffraction. The same system could also undertake nucleation of protein crystals in droplets [113,115].

6. Conclusions

Droplet microfluidics has attracted increasing attention as one of the most promising techniques to realize
high-degree integration and to offer more flexibility in a microfluidic system.

Manipulation of droplets is among the most important issues in this field. When considering the most widely employed approaches for droplet manipulation, both hydrodynamic and EHD stresses have inherent disadvantages (i.e. inflexibility in the control of a single droplet for hydrodynamic stress and complicated fabrication of microfluidic chip for EHD). However, approaches based on thermocapillary and magnetism have to rely on assistance from some other techniques to manipulate droplets. In this respect, there is still plenty of room for improvement in droplet-manipulation techniques. Fortunately, extensive efforts have been dedicated to fundamental studies of droplets in microfluidic systems, including two-phase dynamics in micro-channels, and a number of novel applications (e.g., chemical and biological) have been developed.

We expect that, in the near future, there will be more progress in investigations involving droplets in microfluidic systems.

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