CHAPTER 1

History and Current Status of Droplet Microfluidics

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1.1 Introduction

1.1.1 Single-phase Microfluidics

Droplet microfluidics emerged in the early 2000s as a sub-branch of microfluidics,[1–5] driven by the need for quantitative high-throughput analysis that is demanded in a wide range of applications spanning from life science research to materials synthesis, the pharmaceutical industry and environmental monitoring and assessment.[6–9] The early studies in fundamentals and applications of droplet microfluidics were summarized in several excellent review articles.[10–12] The field has seen an explosive growth over the past decade, which is evidenced by the number of review articles published since 2010. There have been 64 review articles with a search for droplet microfluidics in the title based on Web of Science. This chapter is not meant to provide a comprehensive review in a similar manner, instead, it focuses on briefly

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introducing the evolution of droplet microfluidics, the observed barriers that prevent droplet microfluidics being adopted as an enabling tool for applications and the motivation of this book.

Microfluidics deals with fluid flow, heat and mass transfer at the microscale. Before the emergence of droplet microfluidics, which involves immiscible or partially immiscible fluids to form droplets or bubbles, microfluidics normally works with miscible fluids, which is referred to as single-phase microfluidics for differentiation from droplet microfluidics. Single-phase microfluidics mainly originated from the need to miniaturize chemical analysis systems in the early 1990s with the concept of a miniaturized total chemical analysis system (μTAS) introduced by Manz *et al.* in 1990.[13] Since then, rapid growth was enabled by the advancement in micromachining, in particular photolithography technology, which allows microchannels to be fabricated with high precision.[14] Much of the early work has been dedicated to improving separation performance, for example, realizing capillary electrophoresis (CE) based separation in microchannels.[15,16] The major challenges facing the field of CE at that time included separation resolution, detection sensitivity, throughput of analysis and integration with pre- and post-separation processes. Single-phase microfluidics demonstrated the potential to address all of these challenges in one single platform by leveraging its continuous flow nature and channel network configuration.

Separation resolution of CE is highly influenced by the initial sample plug shape.[16] An ideal initial sample plug should be a narrow rectangular shape to avoid sample dilution before separation; however, it is difficult to realize using traditional sample loading methods. Early work for demonstrating the concept of sample preparation for separation used a simple cross-linked microchannel as shown in Figure 1.1.[15,16] This configuration allows a sample plug to be automatically injected for separation by electrically manipulating the flow rate and flow direction in the intersecting microchannels. Basically,

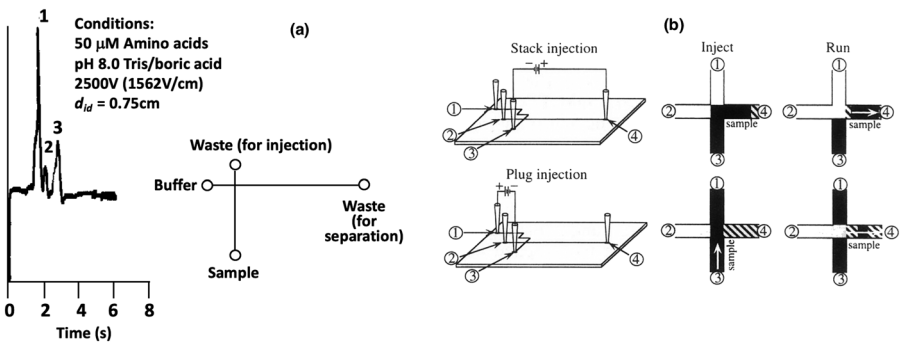

Figure 1.1 Illustration of cross-linked glass microchannels designed for preparing the sample for separation of (a) amino acids; Adapted from ref. 15 with permission from the American Association for the Advancement of Science, Copyright 1993; (b) DNA fragments. Adapted from ref. 16 with permission from the United States National Academy of Sciences, Copyright 1994.

the sample plug was produced by using a buffer flow to 'cut' through a sample flow and can be repeatedly produced for continuous separation presenting a different format of increased throughput. In addition, the detection sensitivity can be increased by integrating pre- or post-separation steps[17,18] and the throughput can also be increased by increasing the number of separation channels for analysis.

1.1.2 Challenges of Single-phase Microfluidics

Explosive growth in the field of single-phase microfluidics did not happen until the introduction of soft lithography technology by Whitesides' team,[19] which enabled networks of microchannels to be fabricated in a polymeric material, polydimethylsiloxane (PDMS), rapidly at a reasonably low cost. It started to find many applications beyond capillary electrophoresis such as cell separation, cell culture and chemical assays.[20,21] The use of elastomeric material also allows the integration of multiple functional components such as valves and metal sensors.[22,23]

The increasing complexity of the channel network for different applications and for integrating multiple different functional components clearly point to the need to involve other disciplines. For example, the design, optimization and operational control of fluid transport in networks of microchannels require mechanical engineering, especially fluid mechanics. The use of different detection methods also draws electrical engineers and physicists in for collaboration. There were numerous innovations developed for a wide range of applications as summarized by many excellent review articles with some commercial systems also available such as the introduction of the LabChip Kit to Agilent 2100 analyzer for DNA fragment analysis by Caliper and Agilent[24] as shown in Figure 1.2 and i-STAT that was later incorporated into the blood-testing product line of Abbott Point of Care.[25]

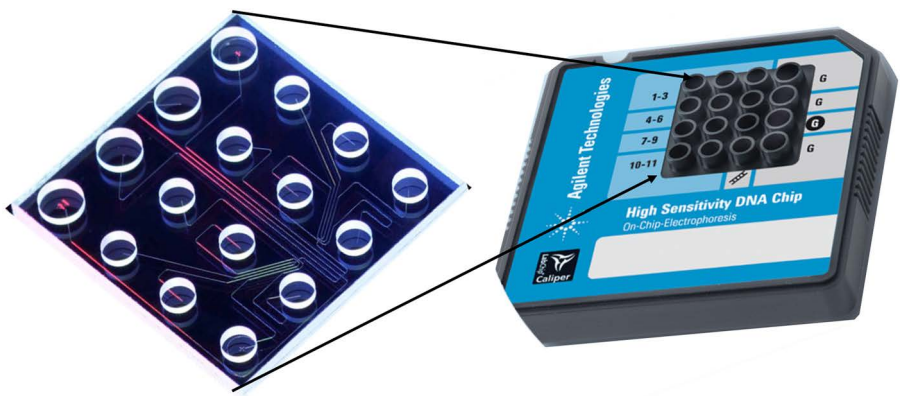

Figure 1.2 LabChip Kit used in the Agilent 2100 analyzer for DNA fragment analysis. Adapted from ref. 24 with permission from American Society for Microbiology, Copyright 2001.

Despite the success of single-phase microfluidics in both knowledge and technological development, some fundamental limitations became very noticeable and discouraging. First, mixing is mainly dominated by molecular diffusion due to its laminar flow nature, which is characterized by Reynolds number that compares inertial force with viscous force. The Reynolds number of microfluidics is typically below one due to its small dimensions. Many efforts have been made to induce convection to enhance mixing such as those by introducing different channel features or external fields as shown in Figure 1.3a and b separately.[26] Both of them complicate the fabrication and operation of the microfluidic devices.

Second, cross-contamination between different samples, which is almost prohibitive for life science research,[27] often occurs because of the challenge to compartmentalize each reaction. Third, the throughput of analysis of a microfluidic device normally increases linearly with the number of channels and thus the device footprint, which mitigates the advantages of microfluidics for miniaturization. In addition, the increase of channel complexity also raises the risk of fabrication failure and operational uncertainties. Lastly, the commonly used material for rapid prototyping of microfluidic devices is PDMS, which is porous by nature and presents many problems for quantitative analysis. For example, PDMS has compatibility issues with fluorescent dyes such as rhodamine B, which has been successfully used for temperature measurement in glass-based microfluidic devices.[28] Rhodamine B adsorbs to and into PDMS causing errors in imaging and temperature measurements.[29] In addition, its

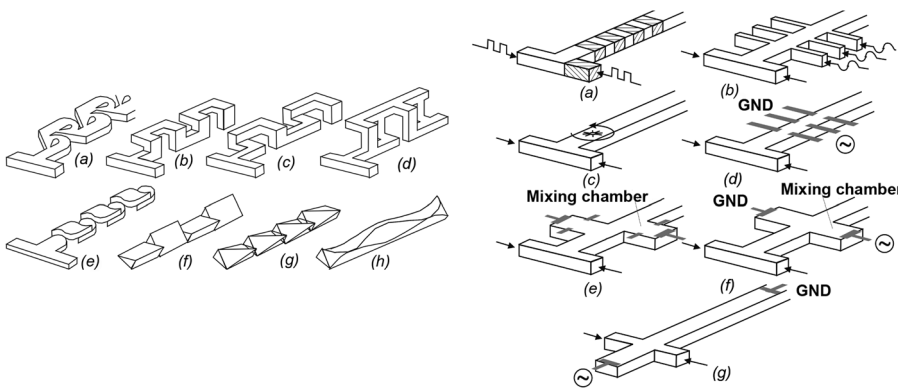

Figure 1.3 Micromixer designs that enhance mixing by varying channel geometries (a) and using external fields (b). Adapted from ref. 26 with permission from IOP Science, Copyright 2005.

surface charge is not stable,[30] which causes unstable flow pumping if an electrical driving flow that relies on the surface charge such as electroosmotic flow is used.

These challenges call for the need to develop new methods that can increase throughput without linearly increasing the device footprint, can compartmentalize each sample process to minimize cross-contamination, and can minimize the dependence on surface charge and properties. The so-called Quake's valve[23] invented in the early 2000s dramatically increased the throughput of analysis to as high as 1 assay per second by integrating thousands of on-chip valves into one single chip[31] as shown in Figure 1.4. Each on-chip valve is controlled by an external valve and thus the control system is complicated and bulky in general for microfluidic chips integrated with a large number of valves. Although the surface compatibility issue is not addressed, each sample process can be compartmentalized using multiple valves, and three valves operating in series also work as a pump, which makes it a powerful platform for the high-throughput analysis demanded by many areas. In addition, Quake's valve does not need to use electroosmotic pumping, which makes it less sensitive to the unstable surface charge.

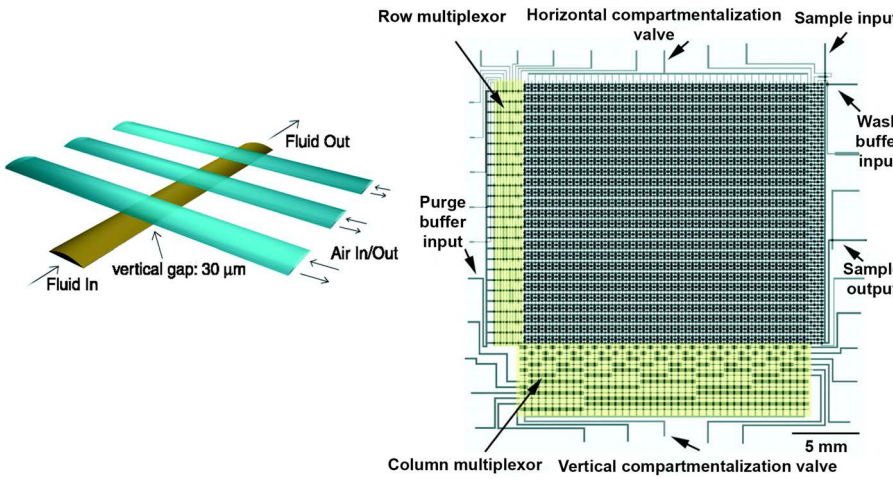

Figure 1.4 (a) An illustration of an elastomeric peristaltic pump. Adapted from ref. 23 with permission from the American Association for the Advancement of Science, Copyright 2000. (b) A mask design for the microfluidic chip that contains an array of 25 × 40 chambers, each of which has a volume of 250 pl. Adapted from ref. 31 with permission from the American Association for the Advancement of Science, Copyright 2002.

1.1.3 Emergence of Droplet Microfluidics

Another alternative to Quake's valve for compartmentalizing sample processes without compromising the throughput was also developed by Quake's group, which employs water-in-oil or oil-in-water emulsions to compartmentalize each sample operation[1] as shown in Figure 1.5. This method was called two-phase droplet microfluidics.

These emulsions can be produced with high monodispersity benefited by the confinement of microchannels at kHz formation rates,[12,32,33] which offers the potential to achieve a magnitude higher throughput than single-phase microfluidics. With good wetting conditions – meaning that the drop fluid and the carrier fluid preferentially wet the chip surface – drops can be fully encapsulated by the carrier fluid, which makes it less sensitive to chip surface properties.[4,34–37] Three-dimensional motion is present in droplets,[38,39] which addresses the slow mixing problem typical of single-phase microfluidics. Integration of multiple droplet manipulation functions such as formation, fission, fusion and sorting is also achieved by a few pioneering studies as shown in Figure 1.6. This platform demonstrates the potential of droplet microfluidics serving as an enabling technology for high-throughput screening analysis.[5]

Driven by its promise and potential to serve as an enabling tool for high-throughput analysis, an exponential growth has been observed in both the fundamental understanding of multiphase flow and technological developments towards various applications spanning from protein crystallization, single cell analysis, gene expression, nanomaterial synthesis and manufacturing particles.

Fundamentally, the understanding of flow competition in terms of its impact on droplet formation and manipulation has been largely improved.[40] Many physical models have been developed describing droplet formation, merging, splitting, sorting and trafficking. The fluid patterns within

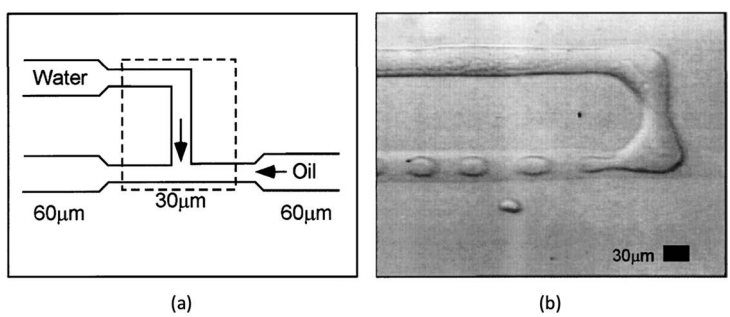

Figure 1.5 (a) An illustration of the channel dimensions of a T-junction droplet generator and (b) an image of the generation of water droplets into the oil stream. Adapted from ref. 1 with permission from the American Physical Society, Copyright 2001.

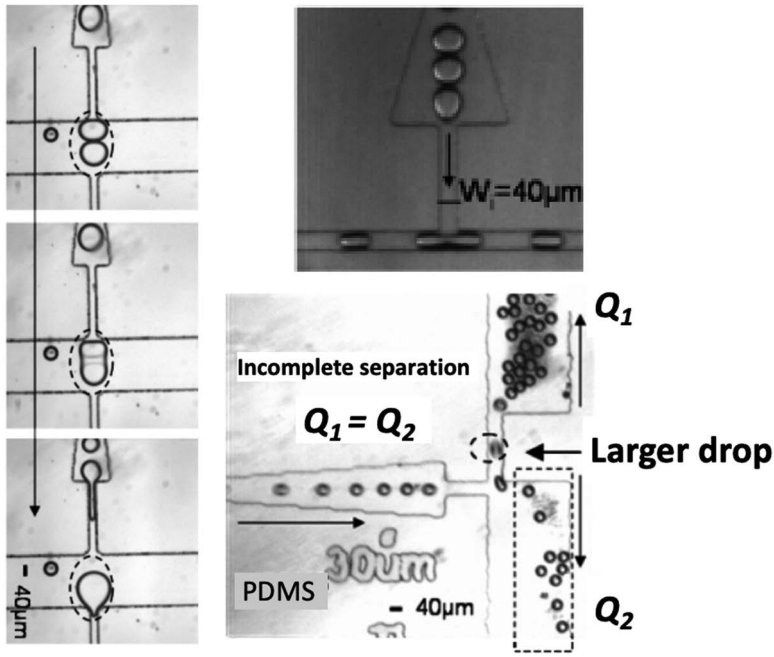

Figure 1.6 Images showing passive droplet fusion, fission and sorting, which were integrated into one single microfluidic chip. Adapted from ref. 5 with permission from the Royal Society of Chemistry.

and outside droplets are also revealed by microparticle velocimetry techniques.[41–43] The impact of surfactant on stable droplet manipulations is also appreciated.[44] These models and the improved understanding of droplet transport are impactful on the design, optimization and operation of droplet microfluidic devices. Technologically, many techniques have been developed[45–49] to manipulate droplets to meet the needs of different applications. In general, the manipulation techniques are categorized as passive and active methods. Passive approaches rely on the variation of channel geometries and flow rates to manipulate droplets.[5] Such approaches in general tend to complicate the channel network and are prone to pressure fluctuations and channel defects. Active methods, however, could work with simple geometry with the cost of integrating external components in both fabrication and operation. Each approach has advantages and limitations.

Recently, a novel active approach has been developed that aimed at leveraging the advantages of both passive and active approaches while minimizing their limitations.[50–52] It eliminates the need to integrate external components as most active methods do and could work with simple geometries to manipulate droplets on-demand, lowering the risk of failures due to fabrication and operational uncertainties. It utilizes image

analysis to identify droplet location, which is used as the feedback to actuate the applied pressures to manipulate droplets based on theories of fluid mechanics.[50–52] It has been demonstrated that the system is able to generate, split, merge, sort and store droplets as demanded using simple T-junctions. This active approach has the potential to serve as the basic building blocks needed for reconfigurable modular systems. For example, each functional component such as a droplet generator, droplet splitter or droplet merger can be a module working with a simple geometry such as a T-junction and different modules can be integrated *via* a connector. Users can select the modules based on their needs and build a system with confidence without the need to understand how each module is developed. This is analogous to building an electrical circuitry without the need to understand how resistors and transistors are developed.

1.2 Challenges and Opportunities of Droplet Microfluidics and Motivation of this Book

Despite its successes and potential as an enabling technology for high-throughput analysis,[53] the expected adoption of droplet microfluidics by applications and associated industries has not yet been seen, which is due to a number of factors that are categorized into two groups.

1.2.1 Interdisciplinary Nature

Droplet microfluidics is interdisciplinary by nature with fundamental roots in physics, chemistry, materials and engineering and finds applications in a wide range of areas such as drug discovery, life science research, material synthesis and environmental monitoring. Its interdisciplinary nature presents a large barrier between technology developers and application users as well as opportunities for collaboration. Each application presents unique requirements for droplet functions that are significantly different from others and thus prevent generic designs from being employed across different applications. For example, single-cell analysis requires a droplet to be small in order to isolate one single cell in one droplet, while tissue engineering requires large droplets to carry large tissues such as the embryoid body of a few hundred micrometers. The single cell droplet microfluidic chip cannot be simply expanded to a chip for tissue engineering by increasing channel dimensions or varying flow rate ratios. It involves a package of fundamental and technological developments. Technology developers who focus on advancing scientific understanding and developing techniques for droplet manipulations are not fully aware of real problems encountered in applications. Application users are discouraged by the complex design and integration of droplet functions. A small change in the application requires the entire channel network to be redesigned, which often is beyond the users' expertise. Collaborations between application users and technology

developers of droplet microfluidics through deep integration of their expertise, knowledge, methods and data are critical to fully realize the enabling potential of droplet microfluidics. This book aims to draw contributions from different application areas formulating a platform to facilitate the dialogue between technology developers and application end users and potentially stimulate the development of strategies to break the barrier. It is difficult to include all kinds of applications in one book, instead, the applications where droplet microfluidics has been successfully used and demonstrate great potential to be employed are considered, including: gene analysis (Chapter 4), since cell gene analysis (Chapter 5), integrative functional analysis (Chapter 6), single cell functional analysis (Chapter 7), synthetic biology (Chapter 8), tissue engineering (Chapter 9) and manufacturing polymer particles (Chapter 10).

1.2.2 Technological Improvements Towards an Enabling Tool

The robustness of droplet manipulation is critical for droplet microfluidics to serve as an enabling tool for various applications and is influenced by many factors:

(1) Chip material. Glass-based materials are solid and there are many methods available for modifying their surface to be hydrophilic and hydrophobic for droplet applications. However, they are in general expensive with ~$500 per chip sold by Dolomite.[54] PDMS has been widely used for rapid prototyping purposes due to its low cost in fabrication and superior optical properties; however, its use for commercially viable products requires extra attention. First, it is incompatible with some fluorescent dyes such as rhodamine B[29] and small molecules[55] that adsorb to and into PDMS. As a result, its surface and optical detection properties would change and the concentration of the chemicals in the sample solution would also change. Second, its softness, which was leveraged as an advantage in fabrication and integration with other components, causes low robustness on droplet manipulation under pressure driven flows because of its deformation.[56] Third, PDMS swells when in contact with most oils. Although the impact could be minimized by saturating PDMS with an oil,[57] the resulting channel height could vary between different batches of fabrication and between different oils. Consequently, the accuracy of droplet manipulation suffers especially under pressure driven flows as the resulting flow rate is inversely proportional to the cube of the channel height such that small changes in the channel height could lead to large deviations in the resulting flow rate from that designed.

(2) Surface property. For stable droplet manipulation, the drop fluid and carrier fluid need to preferentially wet the chip surface.[58] For

example, a hydrophobic chip surface is ideal for making water-in-oil droplets. This could be achieved through a combination of surface modification and use of surfactant; the latter of which will be discussed later. Both hydrophilic and hydrophobic glass based microfluidic chips are available in Dolomite, with the latter being more expensive in general, likely due to the need for surface modification. PDMS is hydrophobic by nature with a water contact angle of $> 100^{\circ}$[59] and turns hydrophilic *via* plasma bonding, which needs to be modified for making either water-in-oil or oil-in-water droplets. Stable droplet formation requires the chip surface to be highly hydrophobic or hydrophilic. To make water-in-oil droplets, PDMS needs to be highly hydrophobic, which has been realized by baking the bonded PDMS chip[4,60] and coating the surface with Teflon based glass surface treatment (Aquapel, PPG Industries).[61] Many methods have also been developed to make PDMS highly hydrophilic,[62] which is suitable for making oil-in-water droplets.

(3) Surfactant. Surfactant is used to prevent coalescence of droplets so that a large number of droplets can be produced and stored for further analysis. Therefore, it is critical to use surfactant for any practical application. Although many types of surfactants have been developed and applied to make emulsions,[44] different surfactants are needed to meet the new needs arising from different applications. In addition, the use of surfactant raises challenges to the design and optimization of droplet microfluidic chips because it influences droplet manipulation. For example, the size of surfactant would influence droplet formation[63] and the performance of the surfactant is also temperature dependent.[44] These effects must be accounted for in the design and operation of droplet microfluidic devices.

(4) Fluid properties. Droplet manipulation is highly dependent on the fluid properties of the fluid pair such as their viscosity contrast and the interfacial tension. Most models describing droplet formation are developed based on Newtonian fluids with a constant viscosity that limits their use in designing droplet microfluidic chips involving non-Newtonian fluids such as blood and some polymers. In addition, interfacial tension is mostly treated as a constant, which is not an ideal assumption for the applications involving temperature variation such as polymerase chain reaction (PCR) or involving processes that are competing with the surfactant kinematics.

(5) Droplet screening and sorting. Droplet manipulations mainly include formation, splitting, merging and sorting, which have all been achieved passively and actively as summarized by many review articles. Although each manipulation is challenging, sorting might be the most challenging in the sense that it is like finding a needle in a

haystack. Droplet microfluidics is featured to be able to generate millions of droplets within minutes. For applications, reagents of interest such as cells are only encapsulated into a small portion of the droplets that require effective screening and then sorting methods to identify and isolate the droplets with reagents for further analysis. Fluorescent imaging is likely to be used to identify the droplet of interest because many assays are developed based on the availability of fluorescent dyes. Early studies used fluorescent dyes to index the droplets for identification purposes.[64]

Passive sorting works on the principle of applying a bias to constantly differentiate droplets based on a sorting parameter. Active sorting uses a mechanism to manipulate the motion of the droplets combined with a means to detect the sorting parameter. Passive methods are limited to sorting droplets based on size. The primary working principle is based on the drag force experienced by different sized droplets.[65,66] Electrical based schemes, such as dielectrophoresis, are the primary method for manipulating droplets in active sorting, which are also combined with other detection methods for screening such as capacitive sensors and optical detection systems.[45] In addition, surface acoustic streaming has also been used to deflect droplets into sorting channels.[67] Recently, a new active approach has also demonstrated its capability for sorting droplets with the assistance of imaging and feedback-loop control.[50–52]

1.3 Summary

Droplet microfluidics holds tremendous potential to serve as an enabling tool for high-throughput quantitative analysis, which is in high demand in many areas such as life science research, drug discovery, personalized medicine and material synthesis. However, its adoption by application end users and prospective industries as an enabling tool has been limited despite the numerous innovations that have been developed. Besides the technical challenges of manipulating droplets including the choice of chip materials, the control of chip surface and fluid properties, the availability of surfactants, and the lack of effective methods for droplet screening and sorting, a few other factors have also contributed to the slow adoption, which include the discipline barriers between technology developers and end-users and the fact that each application has distinct different needs that require new technological developments and fundamental understanding of droplet microfluidics. This book aims to draw contributions from technological developers and application end-users to stimulate collaborations between them and thus facilitate the adoption of droplet microfluidics as an enabling tool.

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