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J. Michael Köhler
Brian P. Cahill *Editors*

Micro-Segmented Flow

Applications in Chemistry and Biology

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Editors

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Preface

During the last dozen years, droplet-based microfluidics and the technique of micro-segmented flow have been evolving into a key strategy for lab-on-a-chip devices as well as for micro-reaction technology. The unique features and advantages of these technologies with regard to the generation and manipulation of small liquid portions in microsystems have attracted widespread attention from scientists and engineers and promise a large spectrum of new applications. The steep increase of scientific interest in the field corresponds to a quickly rising number of publications and to the increasing importance of the field for numerous scientific conferences. Among them, the CBM workshop on miniaturized techniques in chemical and biological laboratories has dealt with droplet-based methods and micro-segmented flow since 2002. In particular, the sixth workshop—held in Elgersburg/Germany in March 2012—focussed on recent developments in micro-segmented flow. This meeting highlighted the progress of the field over the past few years and reflected a well-developed state in the understanding of droplet-based microfluidics, segment operations, in the development and manufacture of devices and in their applications in chemistry and biotechnology. The focus of the meeting on the state-of-the-art in research and development in the science, technology and application of micro-segmented flow proved an opportune occasion for a summarizing description of the main aspects of *Micro-Segmented Flow* in the form of this book.

The authors and editors of this book understand their writing as a mission for giving a representative overview of the principles and basics of micro-segmented flow as well as a description of the huge number of possibilities for processing micro-fluid segments and their applications in chemistry, material sciences as well as in biomedicine, environmental monitoring, and biotechnology. So, the book is divided into three parts: the first part introduces the fascinating world of droplet and segment manipulation. The described methods range from droplet handling by surface forces and light to electrical switching and chip-integrated systems and to sensing of the presence and content of micro-fluid segments. In the second part, the application of micro-segmented flow in the synthesis and operation of micro and nanoparticles is chosen as a typical example of taking advantage of micro-fluid segments in chemical technology. Beside the large spectrum of applications in the preparation of new and homogeneous materials, the potential of micro-segmented flow for the screening of nanoparticle compositions, shapes, and sizes by

combinatorial synthesis is shown by the example of plasmonic nanoparticles and the tuning of their optical properties. Finally in the third part, two important aspects of miniaturized cell cultivation and screenings have been selected for demonstrating the power of micro-segmented flow in biological applications. In both of these chapters, the use of micro-segmented flow for the determination of highly resolved dose/response functions for toxicology, for the characterization of combinatorial effects in two- and three-dimensional concentration spaces and for the application of droplet-based methods and micro segmented flow in the search for new antibiotics are reported.

All authors are active researchers in the field of micro-segmented flow. The chapters follow the concept of connecting a review-like overview of the specific topics with a report on recent examples of the researcher's own research. So, it is expected that the reader will find a very informative survey of the most important aspects and an authentic introduction into the fastly developing and fascinating world of segmented-flow microfluidics.

Ilmenau, April 2013

Contents

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Variables

A	Surface area
A_p	Projected area
A_0	Area between the S11 and S11 = 0 for a loaded sensor
A_l	Area between the S11 and S11 = 0 for an unloaded sensor
C	Capacitance
C_a	Capillary number
C_w	Wall capacitance
C_m	Medium capacitance
C_p	Parasitic capacitance
E	Surface energy
E_0	Initial surface energy
E_∞	Initial surface energy
F	Force
F_B	Buoyant force
F_d	Drag force
F_{DEP}	Dielectrophoretic force
F_{flow}	Force exerted on one segment
F_x	Force in the x direction
F_W	Weight
$F\gamma$	Maximum force due to the change in surface area
G	Geometrical correction parameter
J	Current
J^e	External current density
Q	Electric charge
Q_j	Current source
Q_0	Quality factor for an unloaded sensor
Q_l	Quality factor for a loaded sensor
R	Droplet radius/particle radius
R_a	Droplet radius (case a)
R_b	Droplet radius (case b)

R_m	Medium resistance
R_e	Reynold's number
S_{11}	Reflection coefficient
$SI_{(S11)}$	Reflection index
U	Voltage
U_{ec}	Electrochemical potential for particle charging
U_{RMS}	RMS voltage
V	Volume
V_s	Volume of sphere
V_{cuboid}	Volume of cuboid
W	Work done by the electric field
W_0	Width at half maximum for an unloaded sensor
W_l	Width at half maximum for a loaded sensor
X	Cell constant
Y	Cell constant
Z	Impedance
$\|Z\|$	Impedance modulus
$\|Z\|_\theta$	Impedance modulus at a particular phase value
a	Channel width
b	Distance between electrodes
c	Hole depth
d	Hole diameter
d_w	Thickness of the wall
e	Elementary charge
f	Frequency
f_θ	Frequency at a particular phase value
f	Resonant frequency for an unloaded sensor
f_l	Resonant frequency for a loaded sensor
f_{CM}	Clausius-Mosotti factor
g	Acceleration due to gravity
h	Channel height
h_s	Cuboid height
k_1	Experimentally determined factor
k_2	Experimentally determined factor
l	Length
m	Mass
n	Unit normal
u	Mean velocity of the outer fluid
u_∞	Free-stream velocity
u_+	Mobility of the positive species
u_-	Mobility of the negative species
z	Number of elementary charges
γ	Surface tension
ε	Dielectric constant

ε_0	Permittivity of free space
ε_r	Relative dielectric constant
ε_w	Dielectric constant of the wall
$\varepsilon_{(\infty)}$	Dielectric constant at high frequency
$\varepsilon_{(0)}$	Dielectric constant at low frequency
ε^*	Complex dielectric constant
ε'	Real part of the complex dielectric constant
ε''	Imaginary part of the complex dielectric constant
κ	Curvature
μ	Viscosity
θ	Phase angle
ζ	Ratio of hole diameter to channel height
ρ	Density
ρ_+	Charge density of the positive ion species
ρ_-	Charge density of the negative ion species
σ	Conductivity
τ	Relaxation time
ξ	Drag coefficient
ω	Radial frequency

Chapter 1
Introduction

Brian P. Cahill

1.1 Micro Segmented Flow: A Challenging and Very Promising Strategy of Microfluidics

When scientists and engineers started to realize the idea of the Lab-on-a-Chip, they followed the vision to transfer the power and success of miniaturized systems from solid state electronics into the world of chemistry and molecular biology. The transport and processing of molecules inside highly integrated networks of fluid channels should be controlled in analogy to the transport of electrons through electronic networks and used for powerful analytical procedures, for molecular information management as well as for the synthesis and optimization of new molecules and molecular nanomachines. But during the research on the realization of complex microfluidic systems and experiments guiding homogeneous fluids through microchannels it became more and more clear that this analogy was wrong, that this vision was a delusion.

But, the wrong analogy was only a partial fallacy. The most powerful basic concept behind miniaturization in solid state electronics as well as behind microfluidics is the functional patterning, the hierarchical subdivision of space. It is the same principle which we always observe in living nature and which creates the huge wealth of shapes and structures at the different size scales in the world of organisms. All of the unbelievable plurality of structures and functions in living beings is based on one absolute undispensible concept: fluidic compartmentalization.

The formation of cells is the most fundamental principle of living nature, and liquid compartmentalization is continued in the internal compartmentalization of eucaryotic cells by cell organelles or by the formation of organs and lumens in the development of multicellular organisms. The separation of a small volume from the

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environment, the subdivision of space into well-defined small units, the partial decoupling of the cell's internal chemistry from the outside conditions and the variation of chemical and biomolecular processes between these compartments have been the essential preconditions for the evolution of life. The generation of droplets and fluid segments in micro fluidic devices was driven by the search of methods for controlled manipulation of small liquid portions, but was not primary motivated by the analogy of liquid compartmentalization in nature. But, the principle of formation, controlled transport and processing of such liquid compartments is an obvious analogy

This book is dedicated to the principle and application potential of micro segmented flow. The recent state of development of this powerful technique is presented in nine chapters by active researchers in this exciting field. In the first section, the principles of generation and manipulation of micro fluid segments are explained. It gives the fundamentals of the fluidic behaviour of micro droplets and microfluidic segments and explains the possibilities for control and reliable manipulation of the liquid compartments. In the second section, the micro continuous-flow synthesis of different types of nanomaterials is shown as a typical example for the use of advantages of the technique in chemistry. These examples show how the specific advantages of transport conditions in segmented fluids can be used in order to improve the conditions for continuous-flow synthesis procedures and for improving the quality of products. In the third part, the particular importance of the technique of micro segmented flow in biotechnical applications is presented demonstrating the progress for miniaturized cell cultivation processes, for cell biology and diagnostics and sequencing as well as for the development of antibiotics and the evaluation of toxic effects in medicine and environment.

There are three main aspects of the use of micro fluid segments in technology:

1. Process homogenization by control of transport processes and realization of highly reproducible local mass and heat transfer conditions ("fluidically determined process homogenization")
2. Subdivision of process volumes in order to generate high numbers of independent process spaces ("fluidically defined separate micro reactors")
3. Interface management by fluidically controlled interaction of liquid compartments ("fluidically designed interface processes").

The first aspect is mainly used in micro reaction technology. It allows the implementation of micro-continuous flow processes with very high homogeneity. These processes are marked by very high rates of mixing and heat transfer as well as by an ultimate narrow distribution of residence times. In addition, the pattern of fluid motion inside micro fluid segments is reproducible. In consequence, it can be expected, that each volume element experiences the same "process history". This quasi-perfect homogenization of all transport and reaction processes in all volume elements means a ultimate step in the quality of chemical engineering in continuous flow processes.

The second aspect concerns the experimental realization of high, but ordered diversity. This aspect is of large interest for combinatorial processes, screenings, variation and investigation of process parameters and for the realization of two- or

higher-dimensional concentration spaces. The automated subdivision, the addressing and separate processing of individual fluid segments is, for example, very promising for combinatorial chemistry, for high-throughput diagnostics, for pharmaceutical screenings and for toxicological investigations.

The third aspect relates to the spatial control of interface management. In contrast to suspensions and emulsions, which consist of statistically distributed volume elements, the micro segmented flow realizes well-defined spatial relations between the single liquid compartments, between different types of liquids and between the liquids and the wall. The ordered processing of fluid segments correlates with an ordered transport and processing of interfaces. This is very important for nearly all types of phase-transfer processes and for operations with micro and nanophases. So, the micro segmented flow is, for example, a very promising tool for the synthesis, modification and manipulation of nanoparticles.

The following chapters will introduce us to the fascinating world of micro droplets and fluid segments, will explain the principles of microfluidic functions, describe designs and realization of fundamental devices and give examples for important applications reaching from inorganic chemistry, over organic materials to biological systems.

Part I
Generation, Manipulation and Characterization of Micro Fluid Segments

Chapter 2
Droplet Microfluidics in Two-Dimensional Channels

Charles N. Baroud

Abstract This chapter presents methods for two-dimensional manipulation of droplets in microchannels. These manipulations allow a wide range of operations to be performed, such as arraying drops in two-dimensions, selecting particular drops from an array, or inducing chemical reactions on demand. The use of the two-dimensional format, by removing the influence of the channel side walls, reduces the interactions between droplets and thus simplifies droplet operations, while making them more robust. Finally, the chapter presents further developments on droplet microfluidics without a mean flow of the outer phase.

2.1 Droplets in Linear Channels and on Two-Dimensional Surfaces

The miniaturization of fluid handling tools is a process that has greatly evolved through a large number of independent routes. From pipetting robots or ink-jet printers that can manipulate sub-microliter volumes at high speed, to the formation and transport of liquid segments in straight tubes, several criteria have been considered for determining the optimal approach. Indeed, the robots provide precise and programmable control of a sequence of individual pipetting events and are therefore conceptually simple to program. In contrast, producing a train of liquid segments in a straight tube requires up-front planning, in order to keep track of where the different segments end up, but yields a robust and contamination-free environment for manipulating very large numbers of individual reactors. This tradeoff between the flexibility of programmable machines and the robustness and speed of confined geometries re-appears in more exotic microfluidic tools. In this context again, two independent approaches have also been proposed based on micro-fabricated devices.

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The first approach grew out of the micro-electronics community where a vast knowledge was already available for producing electronic components and managing them. This work has lead to the development of so-called "digital microfluidics", where droplets are produced and manipulated on the surface of a solid substrate. These operations take place through surface stresses, applied for example by an electric field [1, 2], differential heating [3, 4], acoustic waves [5], etc. These stresses have been shown to provide basic operations such as drop production, merging, division, or the mixing of the drop contents. In this technique, the position and movement of each drop can be controlled at every moment, so that the user can program the device operation by software. This implies that a generic chip design can provide any number of different functionalities, with the possibility to the re-program it in real time. However, practical implementations of this platform have typically remained limited to a small number of droplets.

In parallel with advances in digital microfluidics, a large body of work has shown that droplets can be generated and transported at high throughput in microfabricated channels [6–8]. These channels can be produced at much lower cost than surface manipulation chips and they typically operate in a passive way, thus displaying excellent robustness and simple operation procedures, in addition to providing a controlled closed environment within the microchannel. Here again, basic tools have been demonstrated for droplet sorting [9, 10], coalescence [11, 10], mixing [12], as described in several recent review articles that describe the state of the art from the applications or fundamental points of view [13–17].

This comparison between the two approaches yields a panorama that shows that each is suitable for a different kind of experiment and that the overlap between the two is very small. The advantages and disadvantages of each method have lead to application areas that are very different for each of the two approaches: digital methods are well suited for experiments that require a high level of control with a low throughput; they have been applied, for example, for long term incubation of biological samples for cell cultures [18]. In contrast, microchannel methods are suited for statistical studies that require little real-time manipulation but a large number of samples, such as the "digital" Polymerase Chain Reaction (PCR), where an initial sample is divided into a large number of subsamples, such that each droplet contains a single DNA strand, before thermocycling (e. g. [19]).

Recent work however has aimed to bridge the gap between the two approaches, namely by developing ways in which microchannel methods can mimic the functionalities of digital microfluidics methods. This includes, for example, the creation of stationary arrays of droplets within microchannels, for long term incubation and observation, or in order to perform successive operations on these drops. The different approaches have generally relied on the ability to microfabricate fine geometric features within the channels, into which drops can enter but where they get trapped. This allows the drops to be held in known locations, against a mean flow, for long term observation. The use of photo-lithography to make these features allows high levels of parallelization and the implementation of concurrent operations in a large number of independent locations.

Below, we will focus on recent extensions of microchannel techniques that have addressed such possibilities. The chapter begins by describing different approaches that have been proposed, which include quasi-two dimensional and true two-dimensional (2D) devices. Further down, we turn our attention to the use of surface energy gradients for true 2D manipulation in devices with no side walls. First the physical concepts of surface energy and energy gradients are introduced, followed by the practical implementation of "rails and anchors". This is followed by some example realizations that show passive and active 2D droplet manipulation. Finally, the chapter ends with the description of very recent methods to completely remove the need for a mean flow of the carrier phase and a discussion of the possibilities that are afforded by such an approach.

2.2 Generating Droplet Arrays in Microchannels

Several approaches have been proposed to array stationary drops in a microfluidic system, in order to observe their contents for extended periods of time. These designs all rely on using the drops' surface tension, which provides a "handle" to push or hold the fluid segment [20–25]. Indeed, this surface tension resists deformations of the interface and therefore requires a minimum force to be able to squeeze through an aperture. As such, moving a drop from a region of low confinement through a region of high confinement requires the interface to deform and the drop will resist moving through this region. The approaches presented for making the 2D arrays all rely on this principle but differ in the details of how the drops are led to the less confined zones, and how they must squeeze to exit from them.

Several practical principles have guided the published designs, depending on the particular application. In all cases however, the leading desire is to produce a two-dimensional array in order to observe droplets for long periods of time. In this respect, placing the drops in a two-dimensional matrix rather than in a straight line increases the number of drops that can be observed in a given area. Several groups have demonstrated the use of a winding linear channel that is patterned with side pockets where droplets can be held stationary, adapting previous designs that were used to trap solid beads [26], as shown in Fig. 2.1a and b. These devices consist of a linear microchannel in which drops are initially formed and flow in series, so that all of the standard droplet microfluidic methods can be applied to the drops. The trapping region then consists of a specific section in which side pockets are added to the main channel. Droplets occupy them individually or in small groups, until they fill the side pockets. Then later droplets flow past until they reach an unoccupied pocket that they fill. The final result is a channel where the individual pockets are filled with drops from the initial train.

More recently, a more parallelized design was developed by connecting the inlet with the outlet through a large number of parallel channels. Those channels have a variable width, as shown in Fig. 2.1c, so that droplets are held in the wider regions when the flow is stopped or reduced [23]. Having a large number of parallel channels

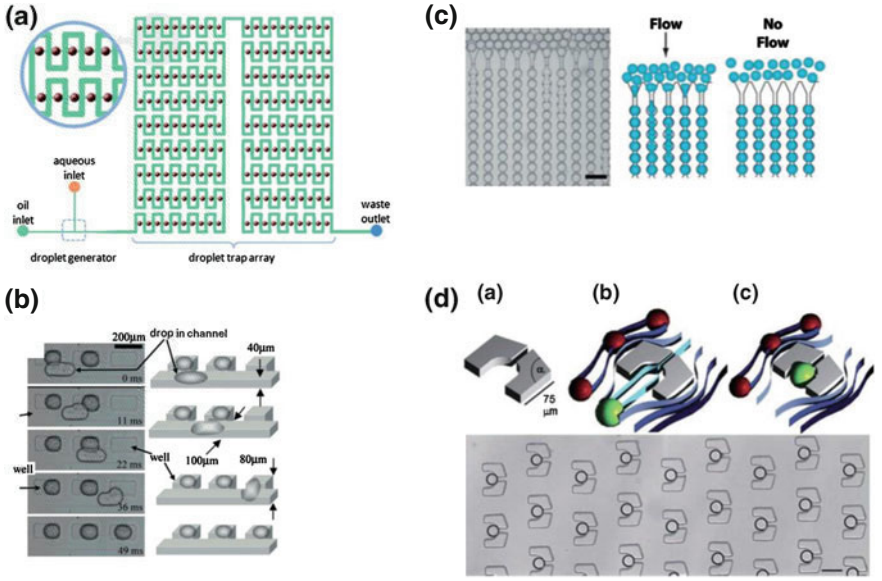

Fig. 2.1 A few trapping devices: **a** from Shi et al. [21]. **b** from Shim et al. [20]. **c** from Schmitz et al. [23]. **d** from Huebner et al. [24]

is favorable to filling them, since once a particular channel fills up its resistance to flow increases, reducing the ability for later drops to enter and leading them to flow into the less occupied channels. Flushing the drops from these channels is however harder for the same reasons: If a channel has been flushed, the carrier phase will preferentially flow through it, leaving the droplets in the full channels unmolested. This device can also be labeled as quasi-2D since droplets interact strongly within each of the individual channels but weakly across channels.

Finally, a truly 2D design was developed by Huebner et al [24], who adapted a design for cell trapping in a microfluidic chamber [27]. In this approach, the side walls are placed very far from the region of interest, such that the droplets are allowed to flow in an open two-dimensional area. This area features micro-fabricated pockets into which a single drop can enter and get trapped. When a drop is trapped it blocks the flow through this region and later drops are directed towards other traps. In this two-dimensional geometry, the behavior of one drop has only a minor influence of others flowing around it and the traps can be filled in a simple and reproducible manner. Moreover, they can be emptied by reversing the flow and the contents can be recovered from the inlet in which they were injected.

These devices all solve the problem of droplet storage in two-dimensional arrays. However they do not offer any method for applying more complex operations, such as guiding the drops into the right spot, selectively removing a single drop, or inducing a droplet fusion for chemical reaction. These operations were treated by later publications [28–30] and will be described below.

2.3 Using Surface Energy Gradients for Droplet Manipulation

The behavior of the devices described above is generally explained by considering the pressure differences in different regions of the microchannels and comparing them with the pressure jump across the drop interface. Indeed, the presence of surface tension introduces a pressure jump (the Laplace pressure jump) across the liquid interface, with the pressure within the droplet being higher than the pressure outside. This pressure jump at every location on the interface is proportional to the surface tension γ and the local mean curvature κ (see [16] for a detailed discussion). Forcing the droplet through an aperture therefore increases the pressure within the drop in the location where it is squeezed since the curvature increases, which requires the external fluid to apply this extra pressure. The drop will therefore remain trapped as long as the external pressure difference is below the pressure necessary to deform the interface.

This reasoning gives a local criterion for the ability to hold a drop stationary, based on the local flow rates and viscosities of the different fluids, as well as on the value of surface tension and constriction size. However, a different way to think about these problems is to consider the surface energy of the drop as it deforms. Indeed, the pressure field arguments above can be reformulated in terms of surface energies and the two approaches are equivalent [16]. The surface energy arguments however provide new insights on droplet manipulation, in particular when considering energy gradients, as discussed below.

Indeed, once a droplet is formed, its volume is essentially fixed (if the effects of dissolution or Ostwald ripening are negligible). However, the surface area of the interface that separates the droplet fluid from the surrounding medium can vary, as the geometry of the drop changes. The minimum surface area for a given volume is a sphere, which is the typical shape of a small, unconfined droplet, and any drop shape that departs from a perfect sphere corresponds to an increase in surface area. Moreover, creating this surface area corresponds to an energetic cost associated with the free energy of the interface, which can be written as

$$E = \gamma A, \tag{2.1}$$

where E is the surface energy, γ is the surface tension, and A is the surface area of the immiscible interface.[1] An increase of surface area therefore leads to an increase of free energy, such that squeezing a droplet between two plates increases the surface energy of the droplet, as shown in Fig. 2.2. This therefore requires a force to be applied by the plates on the droplet.

A more subtle consequence of the dependence of surface energy on confinement occurs when the droplet feels a gradient of confinement . The simplest such gradient is sketched in Fig. 2.3, where a drop can release some of its surface energy by migrating from the left to the right of the confining chamber, as expected intuitively. Indeed, the force that acts on the droplet and generates this motion can be written as the

[1] We will always consider to be constant in this discussion

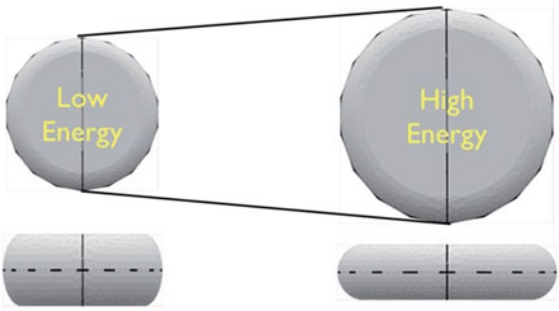

Fig. 2.2 Squeezing a droplet does not change its volume but it does change the surface area of the immiscible interface. Therefore the drop sketched here has a lower surface energy when it is less squeezed (*left*) than when it is more squeezed (*right*)

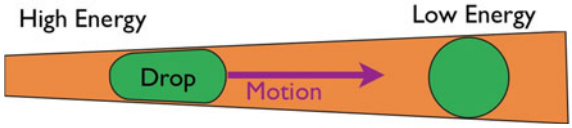

Fig. 2.3 A gradient of confinement corresponds to a gradient of surface energy and therefore a force that pushes the drop from the more to the less confined regions

gradient of the surface energy:

$$\vec{F}_y = -\vec{\nabla} E = -\gamma \vec{\nabla} A \tag{2.2}$$

if the surface tension is considered constant.

This migration of liquid drops as a result of gradients of energy has been known since the 18th century. Indeed, Hauksbee [31] observed that a drop of wetting oil migrates towards the more confined end between two non-parallel plates, thus reducing the total surface energy of the liquid-gas-solid system. This phenomenon has recently been studied in different geometries for both wetting and non-wetting drops and bubbles. For instance, a drop of wetting liquid on a conical needle [32] or inside a pipette cone [33] migrate to minimize the total surface energy, as does non-wetting bubble in a tapered channel [34, 35]. The travel direction for the wetting drop is towards the more confined end, while the non-wetting drop or bubble will migrate towards the less confined end. In the latter case, the migration stops either when the drop reaches a region in which it is no longer confined, i.e. when it becomes spherical, or when it reaches an obstacle that blocks its advance. The first case corresponds to a global energy minimum and the second case to a local energy minimum.

2.4 Rails and Anchors

2.4.1 Principle of Droplet Anchors

In the context of microfluidics, lithography methods allow confinement gradients to be produced locally, for example by etching a small groove in the surface of a microchannel. Therefore if a spherical drop enters a microchannel whose height is

smaller than the sphere diameter, the drop must squeeze and thus depart from its spherical shape. The resulting deformation leads to an increase in its interfacial area and a corresponding increase in free energy. By this mechanism, the drop can store and transport this extra energy as it travels down the channel. Given the chance, it will tend to decrease its surface area in order to reduce its free energy. These confinement variations can be in the form of a local indentation in one of the channel boundaries. The simplest implementation is therefore to make a single hole in the surface of the microchannel and to lead the drops to this position with the outer flow. For a wide range of hole and droplet sizes, this leads to the "anchoring" of the drop at the location of the hole, even when the outer phase is flowing past the drop location, as shown in Fig. 2.4.

Moreover, lithographic microfabrication methods allow the production of complex two-dimensional shapes. Therefore etching a linear groove into the surface of an otherwise flat channel can lead the drops to follow the direction of these so-called "rails". When they are directed at an angle compared with the mean flow, the rail can be used to guide the droplets sideways within the wide section, thus allowing droplet guidance in the absence of rigid lateral walls. As an example, a sinusoidal rail is shown in Fig. 2.5, where drops are shown to follow the wavy path imposed by this etched structure. Naturally, any shape can be drawn in order to create rails of simpler or more complex structures, as we shall see in later sections.

The surface energy landscape for a traveling droplet can therefore be fashioned with a complex pattern of energy barriers and wells which then lead the drop to follow the path of least resistance. In the case of dilute flux of drops, predicting the motion of each of them is relatively straightforward, as we shall see in the sections below. This can be understood by first calculating the magnitude of the forces that come into play on the droplets.

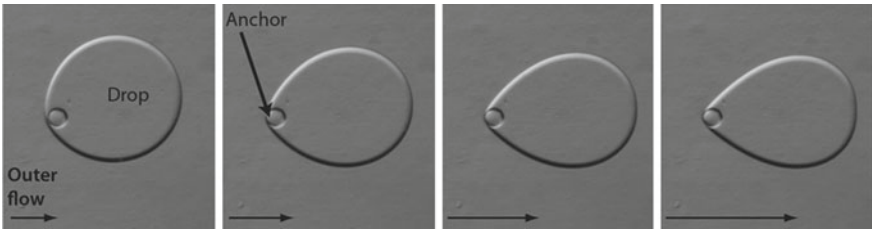

Fig. 2.4 A drop that is initially squeezed between the top and bottom surfaces of a Hele-Shaw cell will be attracted to the location of a local hole in the microchannel wall. As the strength of the outer fluid flow is increased, the drop can deform without detaching from the anchor