Development and characterization of micromixers

Wu, Zhigang

2005

Wu, Z. (2005). Development and characterization of micromixers. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/5413>

<https://doi.org/10.32657/10356/5413>

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DEVELOPMENT AND CHARACTERIZATION OF MICROMIXERS

WU ZHIGANG

SCHOOL OF MECHANICAL & AEROSPACE ENGINEERING
NANYANG TECHNOLOGICAL UNIVERSITY

2005

Development and Characterization of Micromixers

Wu Zhigang

School of Mechanical & Aerospace Engineering

A thesis presented to Nanyang Technological University
in fulfillment of the requirement for the Degree of
Doctor of Philosophy

2005

Abstract

Microfluidics is a new research field focusing on the development of miniaturized devices, which can detect and handle microscopic fluid amounts. With the growing importance of life science, microfluidics is the enabling technology for genomics, proteomics and drug delivery. One of the most important, but little discovered fields of microfluidics, is mixing and transport effects in microscale. In practice, rapid mixing is essential in many of the microfluidic systems targeted for the use in biochemical analysis, drug delivery, and sequencing or synthesis of nucleic acids, among others.

This thesis focuses on mass transport and mixing in microscale. A number of analytical models are formulated to discuss the transport effects in the basic T-mixer. First of all, a two dimensional convective-diffusive model of diluted mixing with a 1:1 ratio is established and solved analytically. This model is then extended to mixing with an arbitrary mixing ratio as well as with multiple mixing streams. Furthermore, Taylor dispersion is considered in the later model. Besides mixing of streams with the same viscosity, mixing between streams with different viscosities are also considered. Hydrodynamic focusing helps to decrease the mixing path and to enhance the mixing effect. An analytical model for hydraulic focusing is also discussed in this thesis. Because of the difficulty of an analytical solution for the case of mixing between streams with different viscosities, numerical simulation is used. Finally, non-linear effects of mixing in a microchannel are discussed.

To verify the theoretical models, a series of fully polymeric prototypes were fabricated through direct laser micromachining. A direct thermal bonding technique was developed for hermetical seal of the device. The experimental results show good agreements with theoretical models. Among the characterization, a quantitative method was offered to evaluate the performance of passive parallel micromixers.

Finally, two novel designs of micromixers were proposed for better mixing. The characterization show high potentials of these two designs. Furthermore, a novel fabrication process for three dimensional channel was offered in the latter design. The technique has been proved as a low cost, robust, simple operation and rapid process for fabrication of microfluidic devices.

To my parents

Acknowledgments

The author wishes to express his sincere gratitude and appreciation to the following people, who had offered invaluable contribution to this thesis:

- Assistant Professor Nguyen Nam-Trung, the author's supervisor, for his sincere encouragements, invaluable guidance, endless patience, and friendship during the past three years;
- Associate Professor Huang Xiaoyang, and Assistant Professor Charles, Yang Chun for their respectable guidance and support;
- My fellow research students, labmates, and technician staff in thermal and fluids lab and many other people helped me with useful discussions, suggestions and experimental support;
- My parents for their deep loves and continuous encouragements.

Nomenclature

β	Viscosity ratio
Δt	Time intervals (s)
γ	Flow rate ratio between the one sheath stream and the sample stream in hydraulic focusing
κ	Dimensionless mixing length
λ	Mean free path (m)
μ	Dynamic viscosity (kg·s/m ²)
ν	Kinematic viscosity (m ² /s)
ϕ	Diffusion flux (kg/ms)
ρ	Density (kg/m ³)
ρ_p	Density of the particle (kg/m ³)
τ_s	Response time of particles (s)
θ	Angle (rad)
$\vec{F}$	External force (N)
$\vec{v}$	Velocity vector
a	Non-linear coefficient /Acceleration (m ² /s)
A, B	Coefficients defined by Fourier analysis

c	Concentration of specie (kg/m^3)
c^*	Normalized concentration
c_0	Solute initial concentration
D	Diffusion coefficient (m^2/s)
D_h	Hydraulic diameter (m)
d_B	Brownian motion displacement of particles (m)
d_p	Diameter of the particle (m)
D_x	Diffusion coefficient along x -axis (m^2/s)
D_y	Diffusion coefficient along y -axis (m^2/s)
f	Disturbance frequency (Hz)
$f(i, j), g(i, j)$	Image intensity arrays
H	Channel height (m)
h	Dimensionless height
i, j, p, q	Indices
I^*	Dimensionless intensity
$I_{\max}$	Maximum intensity
$I_{\min}$	Minimum intensity
k	Boltzmann's constant
K_n	Modified Bessel function of the second kind n -order
L	Mixing path/Channel length (m)
m	Number of serial mixing stages
n	Number of laminae

P' Pressure term ($P' = \frac{W}{u_0 V} \frac{dp}{dx^*}$)

Q_1, Q_2 Flow rates (m^3/s)

R Radius of injection nozzle (m)

r Reaction rate / Mixing ratio/ Dimensionless interface of mixing flow

r^* Dimensionless radius

T Absolute temperature (K)

t Time (s)

U Average flow velocity (m/s)

u Velocity along x direction (m/s)

u^* Dimensionless velocity along x direction

u_0 Reference velocity (m/s)

U_s Velocity lag of particles in fluid (m/s)

W Channel width (m)

Kn Knudsen-number

Pe Peclet number

Re Reynolds number

St Strohal number

MEMS Micro electromechanical system

PC Polycarbonate

PDMS Polydimethylsiloxane

PET Polyethyleneterephthalate

PMMA Polymthylmethacrylate

PS Polystyrene

PT Polyethylene

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Chapter 1

Introduction

1.1 Background

Miniaturization is the recent trend in analytical chemistry and life sciences. In the past two decades, miniaturization of fluid handling and fluid analysis has been emerging to the interdisciplinary research field of microfluidics [1, 2, 3]. Micro electromechanical systems (MEMS) [4] provides the new field of microfluidics with many of the key components such as flow channels, flow sensors, chemical detectors, separation capillaries, mixers, filters, pumps, and valves. All these components can be fabricated with micromachining techniques. Chemical analysis, drug delivery, biological sensing, micro array, DNA sequencing, sample preparation and analysis, cell separation and detection, environmental monitoring and many other applications typically incorporate microfluidic devices. However, microfluidics is still in an early stage of its development. Previously, the fabrication of microfluidics was based on technologies of MEMS. The basic substrate materials were silicon and glass. Recently, arising from the need of low cost and biocompatibility, polymers have been extensively used for making micromixers and other microfluidic devices. A number of polymeric fabrication techniques are readily available. Polymeric bulk micromachining such as hot embossing, injection molding, casting, and laser ablation realized structures in a polymer substrate, while polymeric surface micromachining creates movable polymeric microstructures using a sacrificial layer.

Similar to the miniaturization of microelectronics, the integration of microfluidic de-

vices leads to the development of independent systems on a "chip", that can work alone without a large number of auxiliary utilities, such as systems for Flow Injection Analysis (FIA) [5], Micro Liquid Handling Systems (MLHS) [6], Micro Total Analysis Systems (microTAS) [7, 8, 9, 10]. Such a system is more powerful than a single component. It can carry out many new and complicated functions in parallel. With many integrated components, microfluidics offer attractive features such as parallel processing, remote control, self diagnosis, and other. In this century, bioscience and bioengineering become more important than ever before. These fields give microfluidics a broader stage. Cell patterning, DNA analysis, cell manipulation, genetic sequencing, drug discovery and drug delivery are typical microfluidic applications.

What does microfluidics offer? Firstly, it can reduce costs due to the small size and volume. Small device size leads to small amount of materials, which are processed in the device. This advantage becomes more obvious in analysis, which requires fewer expensive reagents and samples. In many cases, sampling is difficult and may brings many inconveniences. For example, to learn the cloning state of Hepatitis B Viruses (HBV), doctors need to sample more than 5 ml blood from the patients every six months. It is a painful process for many people. Thus, the small sample in microfluidics is a good news for these patients.

Secondly, reactions are safer through the use of small quantities of reagents. Many reactions are dangerous in large quantity. But with a small quantity, these reactions become more moderate and controllable.

Thirdly, small size also makes devices portable. Portability allows investigations outside laboratory environments. Analysis can be carried out everywhere. At the same time, portability also brings real-time processing. Conventional environmental monitor is a slow process, often not real-time. This process cannot detect danger and provide warning on time. Furthermore, small size can reduce human errors and brings high sensitivity. In addition, microfluidics enables massive parallel analysis, which results in higher throughput than a macro system does.

For the reasons above, microfluidics becomes an important research topics. In the past few years, besides the existing journals such as *Journal of Microelectromechanical System*, *Journal of Micromechanics and Microengineering*, more journals were dedicated to microfluidics research such as *Biomedical Microdevices*, *Lab on a chip*, and *Microfluidics*

and Nanofluidics. Traditional journals such as *Analytical Chemistry*, *AIChE journal*, *Analytica Chimica Acta*, *Sensor and Actuators B*, and *Applied Physics Letter* et al. also show huge interests on microfluidics. In the past few years, the number of archival journal papers on microfluidics has been increasing almost exponentially. Nowadays, many conferences of professional societies such as IEEE (Institute of Electrical and Electronic Engineers), ASME (American Society of Mechanical Engineers), SPIE (International Society of Optical Engineering), AIChE (American Institute of Chemical Engineers) have technical sessions on microfluidics [1].

1.2 Mixing in microscale and micromixers

Ho and Tai [11], Brody et al. [12], and Fruman [13] discussed the changes in behavior of fluid flow in microscale. Compared to those in macroscale, the large surface-to-volume ratio in microscale affects the ratio of the different forces in the flow. Surface forces overtake inertia forces, which once were dominant in macroscale. Many effects such as slip boundary, pressure/thermal creep, and electric double layer (EDL) need to be considered [14]. The other significant phenomenon is that the flow is laminar and not turbulent in small scale. The Reynolds number becomes very small compared to that of macroscale.

The above mentioned effects change the behavior of micro flows for mass transport and mixing. Rapid mixing is essential in many of the microfluidic systems targeted for the use in biochemical analysis, drug delivery, and sequencing or synthesis of nucleic acids. Biological processes such as cell activation, enzyme reactions, and protein folding often involve reactions that require mixing of reactants for initiation. Mixing is also necessary in lab-on-a-chip (LOC) platform for complex chemical reactions. Micromixers can be integrated in a microfluidic system or work as stand-alone devices. While good and fast mixing is required in microfluidic applications, effective mixing schemes in microscale are still not well investigated.

Besides micropump, micromixer is another important component in a microfluidic system. However, in contrast to numerous works in micropump [15, 16, 17], few works were done on micromixers [18, 19, 20, 21, 22]. In the past, the importance of micromixers were not well recognized and only few research groups focused on this area.

As an important component of a microfluidic system, a micromixer can be used for the mixing process in a system or as a stand-alone device. The quality of mixing affects the quality of the whole system. Conventional mixing methods are based on stirring or turbulent flow. The small dimension and consequently the low Reynolds number is the crucial problem of mixing in microscale.

1.3 Objectives and scopes

New tools and technologies in microscale help us to explore new effects. Optical diagnosis methods such as micro particle image velocimetry and scalar field measurement based on fluorescent technique have been proved as effective in microfluidics. They are powerful tools for visualizing flows and processes in microscale. The main goal of this thesis is to establish techniques, methods and theories for obtaining better mixing in microscale. The thesis includes theoretical analysis, numerical simulation, setup of experimental instruments, fabrication of prototypes and their characterization. Finally, based on the knowledge from both theory and experiments, two new designs are proposed and tested. Thus, the objectives of the work are:

- Modelling works based on both analytical and numerical methods are to be conducted for better understanding of the mixing and mass transport phenomena in microscale;
- Fabrication of prototypes and development of visualization technique are to be conducted for proving the suitability of the modelling works and for better understanding the physics of the above problem;
- Novel designs of micromixer should be investigated and evaluated for better mixing.

Corresponding to the objectives, the scopes of the thesis are:

1. To determine the optimization parameters for better mixing;
2. To model mass transport and mixing in microscale with analytical or numerical methods;

3. To fabricate the prototype according to the theoretical models utilizing the present fabrication technologies;
4. To set up and calibrate a system for characterizing the fabricated prototype utilizing the present microfluidic diagnosis methods;
5. To design, fabricate and characterize novel micromixers utilizing the knowledge from the above works.

1.4 Organization of the thesis

This thesis consists of seven chapters. Chapter one gives a general introduction to the technology background, objectives and scope of the thesis. Chapter two discusses the state-of-art of mixing theory, the existing micromixers, their characterization as well as fabrication techniques. In Chapter three, analytical modelling of mass transport and mixing in microchannels are presented. Chapter four introduces materials, fabrication, and characterization techniques used in the thesis. Chapter five compares the experimental results with the modelling works in Chapter three. In Chapter six, two novel micromixers were presented and tested. Finally, Chapter seven gives conclusions and outlook on mixing in microscale and micormixers.

Chapter 2

Literature review

This chapter gives an overview survey on micromixing and micromixers from theory, to existing examples, to materials and fabrication techniques, to characterization technologies. Because of the small Reynolds number, the mixing theory in microscale is different from its counterpart in macroscale. There are two main types of mixing in microscale: molecular diffusion and chaotic advection.

Micromixers can be categorized as passive and active micromixers. Passive micromixers are highly dependent on molecular diffusion. The mixing efficiency relies on the increase of the contact surface of mixing streams. The common optimization methods of passive mixers are increasing the contact surface and decreasing the transverse diffusion distance. Recently, researchers found that chaotic advection also can be obtained at low Reynolds number with special channel structures. In active micromixers, external energy is imposed to the mixers to produce field disturbance in a mixing channel or a mixing chamber. Active micromixers are based on a wide range of disturbance schemes such as pressure field disturbance, electrohydrodynamic disturbance, dielectrophoretic disturbance, electrokinetic disturbance, magnetic hydrodynamic disturbance, acoustic disturbance and thermal disturbance.

Few years ago, almost all micromixers were fabricated on silicon or glass substrate using the traditional technique of micro-machine such as wet etching. Recently, with the requirements of biocompatibility and low fabrication cost, polymers were investigated by many researchers.

Characterization techniques are important to develop devices and evaluate new technologies. The evaluation of the performance of a micromixer depends on the accuracy of the characterization technique. In this chapter, the principles of evaluating mixing effect in micromixers are also discussed.

2.1 Theoretical works on mixing

Einstein's theory on thermal motion of molecules [23] is the foundation for diffusion theory. For mixing in macroscale readers can refer to [24]. In macroscale the two common mixing methods are the generation of turbulence [25] and chaotic advection [26, 27]. In a turbulent flow, fluid motions vary irregularly so that quantities such as velocity and pressure show a random variation in time and space. The random movement disperses quickly the mixed components. Chaotic advection can be generated by stirring the flow, which is very effective for small Reynolds numbers. The concepts of splitting, stretching, folding and breaking up are critical for mixing quality.

It is a logical approach to miniaturise mixing concepts of macroscale. Unfortunately, macroscale concepts cannot be applied directly in microscale. In macroscale, chaotic flow can be produced easily through stirring or heating. But in microscale, the Reynolds numbers are often small, and turbulence is not available to enhance mixing. Furthermore, it is often not feasible to incorporate moving components, such as stirrers into micro devices. Thus, new approaches are needed. Diffusion is regarded as one of the solutions.

2.1.1 Basic laws and control equations

According to Fick's law [28], mixing is based on diffusion whose flux ϕ is described as:

$$\phi = -D \frac{\partial c}{\partial x} \quad (2.1)$$

where D is the diffusion coefficient (m^2/s) and c is the specie concentration (kg/m^3). The diffusion coefficient is a fluid property, which is inversely proportional to the viscosity μ :

$$D = C/\mu \quad (2.2)$$

where C is the constant incorporating all other factors of the specie. Generally, the flux is constant in a micromixer. Thus, the mass transport by diffusion is proportional to the contact surface of the two mixed species. The mixing time t is related to the width of the mixing path L [23]:

$$t = L^2/2D \quad (2.3)$$

Equation (2.3) shows that the diffusion time (or the mixing time) depends on the mixing path. Decreasing the mixing path reduces mixing time. On the one hand, the small size of channel in micromixers can improve mixing quality significantly. The only optimization measure left is increasing the contact surface of the mixed streams. On the other hand, if the channel geometry is too small, the fluid molecules collide most often with the channel wall and not with other molecules. Molecular diffusion will decrease quickly and can be neglected [29]. The diffusion process in this case is called Knudsen diffusion. The ratio between the distance of molecules and the channel size is characterized by the dimensionless Knudsen-number [1]:

$$Kn = \lambda/D_h \quad (2.4)$$

where λ is the mean free path and D_h is the hydraulic diameter of the channel structure. The mean free path for gas is given by Cunningham [30]. In liquids, the Knudsen number is so small that it is not as important as in gases. Because the mean free path of liquids is on the order of few Angstrom, diffusion has the same characteristics as in macroscale. Thus, Knudsen diffusion is not significant in liquid. Under standard conditions the mean free path of gases is of the order of nanometers.

Rearranging Equation (2.3), we get the mixing path:

$$L = \sqrt{2Dt} \quad (2.5)$$

which usually gives us a method to estimate diffusion distance. But recently, Ismagilov et al. [31], Kamholz and Yager [32] found that this equation is not correct near the wall of the channel due to nonuniform velocity distribution. They drew a conclusion from their experiments, the equation for the latter situation is:

$$L = \sqrt[3]{2Dt} \quad (2.6)$$

Song et al. [33] did the similar attempt on the scaling law through experimental methods.

As mentioned above, the theory of macroscale cannot be applied directly in microscale. Although turbulent flow is not possible in microscale, it is feasible to achieve similar mixing result with new methods such as chaotic advection. These micromixers are called active mixers. An active mixer is defined as a mixer with external energy input for enhancing mixing.

In micromixers, the other mechanism affecting mixing is convection. Convection exists with diffusion during the mixing process. In a Cartesian coordinate system, the general equation both include convective and diffusive effects in the microchannel has the following form [34]:

$$\frac{\partial c_i}{\partial t} + \nabla(\vec{v}c_i) = D_i \nabla^2 c_i + r_i \quad (2.7)$$

where c_i is concentration of the specie i , t is the time, $\vec{v}$ is velocity vector of the flow, D_i is the diffusion coefficient of the specie i , r_i is the rate of production of the specie i per volume.

2.1.2 Modelling for mixing in microscale

Analytical modelling and numerical simulation are the two basic approaches for describing mixing in microscale. Most of the analytical works are based on Equation (2.7). The equation is a two order partial differential equation with boundary conditions. Besides this equation, the velocity also needs to be solved. There are two methods to obtain the velocity. The first method uses an approximation for velocity profile in the channel. The second method solves the Navier-Stokes equation combined with continuity equation. Unfortunately, the Navier-Stokes equation is not easy to solve using analytical tools. Only small number of special cases can be solved. Thus, the numerical method is usually used for complex cases.

Kamholz et al. [35] used an average uniform velocity and ignored the axial diffusion along the channel to set up a simple model. Later, Holden et al. [36] published their analytical solution under similar conditions. Beard [37] and later comment [38] and response [39] gave an analytical model with the solution in a microfluidic channel. This work assumed a parabolic profile in the channel height direction and a uniform distribution along the width of the channel. To simplify the convection effect that brought by the nonuniform velocity

along the channel height, Beard introduced an effective Taylor dispersion coefficient, which already existed in macroscale [40]. A similar work was done by Kamholz et al. [32] using numerical methods. Further, Beard's approach was extended to model discrete drop mixing in the microchannel by Handique and Burns [41]. Dynamic pulsing is also a method to enhance the mixing. Deshmukh [42] utilized commercial computational fluid dynamics (CFD) tools to model his pulsate micromixer. Besides straight channel, zigzag channel and three-dimensional channel shape were also investigated using numerical methods [43, 44].

2.1.3 Parameters for micromixers

Operating parameters of micromixers are dimensionless numbers such as Reynolds number Re , Peclet number Pe , and Strouhal number St . The Reynolds number:

$$Re = \frac{UD_h}{\nu} \quad (2.8)$$

represents the ratio between momentum and viscous friction. A high Reynolds number above a critical value (2300 in macroscale) indicates a turbulent flow. In most cases of microfluidics, a low Reynolds number and a laminar flow can be expected. The Peclet number:

$$Pe = \frac{UL}{D} \quad (2.9)$$

represents the ratio between the mass transport due to convection and that of diffusion. Convection is dominated at higher Peclet numbers. The Strouhal number:

$$St = \frac{fD_h}{U} \quad (2.10)$$

represents the ratio between the residence time of a species and the time period of its disturbance in an active micromixer.

2.2 Device examples

In general, micromixers can be categorized as passive micromixers and active micromixers, Fig. 2.1. Passive micromixers do not require external energy, the mixing process relies entirely on diffusion or chaotic advection. Passive mixers can be further

Figure 2.1: Classification scheme for micromixers

categorized by the arrangement of the mixed phases: parallel lamination, serial lamination, injection, chaotic advection and droplet. Active micromixers use disturbance generated by an external field for the mixing process. Thus, active mixers can be categorized by the types of external disturbance such as pressure, temperature, electrohydrodynamics, dielectrophoretics, electrokinetics, magnetohydrodynamics, and acoustics. With external fields and the corresponding integrated components, the structures of active micromixers are often complicated and required complex fabrication processes. Furthermore, external power sources are needed for the operation of active micromixers. Thus the integration of active mixers in a microfluidic system is both challenging and expensive. In contrast, passive micromixers do not require external actuators except those for fluid delivery. The often simple passive structures are robust, stable in operation and easy to be integrated in a more complex system.

2.2.1 Passive micromixers

Because of its simple concept, passive mixer was one of the first microfluidic devices reported. Due to the dominating laminar flow in microscale, mixing in passive micromixers relies mainly on molecular diffusion and chaotic advection. Increasing the contact surface

Figure 2.2: Parallel lamination micromixer: (a) the basic T-mixer and (b) Y-mixer, (c) concept of parallel lamination, (d) concept of hydraulic focusing.

between the different fluids and decreasing the diffusion path between them could improve molecular diffusion. Chaotic advection can be realized by manipulating the laminar flow in microchannels. The resulting flow pattern shortens the diffusion path and thus improves mixing.

Parallel lamination micromixers

As mentioned above, fast mixing can be achieved by decreasing the mixing path and increasing the contact surface between the two phases. Parallel lamination splits the inlet streams into n substreams, then join them into one stream as laminae. The basic design is a long microchannel with two inlets ($n=2$). According to its geometry this designs are often called the T-mixer or the Y-mixer [35, 32, 31], Fig. 2.2a,b. As a basic design, T-mixer is ideal for investigations of basic transport phenomena in microscale such as scaling law, butterfly effect [35, 31], and other non-linear effects [45].

Since the basic T-mixer entirely depends on molecular diffusion, a long mixing channel is needed. Besides the above mentioned concept of lamination of multiple streams, mixing at extremely high Reynolds number could also results to a short mixing length [46, 47]. A chaotic flow is expected at these high Reynolds numbers. The induced vortices enhance significantly the mixing efficiency. In [46], a Y-mixer made of co-fired ceramic tapes with

a 90°-bend can generate vortices at Reynolds number above 10. At a Reynolds number higher than 30, mixing is achieved right after the bend. A in glass/silicon fabricated T-mixer [47] utilizes Reynolds numbers up to 500, where flow velocity can be as high as 7.60 m/s at a driven pressure up to 7 bars. Under extremely high Reynolds number ($Re=245$, 45 m/s) a fluid flow can also generate high shear to drive very fast circulation in a diamond-shaped cavity close to a straight microchannel [48]. Fast vortices are generated to enhance mixing with multiple inlet streams focused in a circular chamber [49]. In these micromixers, the high velocities on the order of 1 m/s (7.6 m/s in [47]), 10 m/s [49] or even higher (up to 45 m/s in [48]) require high supply pressures. The high pressure (1.0 bar to 5.5 bars in [47], 15 bars in [49]) can be a serious challenge for the bonding and interconnection technologies. The basic T-design can be improved by roughening the channel wall [50] or throttling the channel entrance [51]. At high Reynolds numbers the basic T-mixer can be further modified with obstacles, which generate vortices and chaotic advection. These types are dealt with later.

Simple methods to reduce the mixing path are making a narrow mixing channel [52], realizing parallel lamination with multiple streams [53, 54, 55] (Fig. 2.2c) or with 3-D interdigitated mixing streams [56]. A parallel lamination mixer with 32 streams can achieve full mixing in 15 ms [57]. This mixer type was successfully used in a practical analysis [58]. The flow in micromixers based on parallel lamination are usually driven by pressure, but can also be generated by electroosmosis [59, 60, 61].

Another concept of reducing mixing path for parallel lamination micromixers is hydrodynamic focusing [62]. The basic design for hydrodynamic focusing is a long microchannel with three inlets. The middle inlet is for the sample flow, while the solvent streams join through the other two inlets and work as the sheath flows, Fig. 2.2d. Hydrodynamic focusing reduces the stream width, and consequently the mixing path. The prototype reported in [62] has a mixing channel of $10\ \mu\text{m} \times 10\ \mu\text{m}$ cross section. The sample fluid can be focused to a narrow width by adjusting the pressure ratio between the sample flow and the sheath flow. In the reported experiments, the mixing time can be reduced to few microseconds [63]. Hydrodynamic focusing was used for cell infection as reported in [64].

Table 2.1: Parallel lamination micromixers.

Ref.	First author	Year	Type	Channel width (μm)	Channel height (μm)	Typical velocity (mm/s)	Re	Pe	Materials
[35, 32]	Kamholz	1999	T-mixer	550	25	6	0.3	725	Silicon-glass
[31]	Ismagilov	2000	Y-mixer	90	90	7	0.4	240	PDMS-glass
[65]	Hinsmann	2001	Y-mixer	1000	20	83	1.7	830	CaF ₂ -SU8-metal-glass
[45]	Wu	2004	Y-mixer	900	50	0.27	0.02	150	PMMA
[46]	Yi	2003	Y-mixer	200	200	50-200	80	80000	Ceramic
[47]	Wong	2004	T-mixer	100	50	7000	500	700000	Silicon-glass
[49]	Böhm	2001	Vortex	20	200	10000	200	200000	Silicon-glass
[50]	Wong	2003	Cross-shaped	30	40	5000-10000	170-340	150000	Ceramic
[51]	Gobby	2001	T-mixer	500	300	0.3	0.1	150	n/a
[52]	Veenstra	1999	T-mixer	100	200	0.17	0.023	170	Silicon-glass
[55]	Koch	1999	Parallel lamination	85	5	0.7	0.0035	60	Silicon-glass
[57]	Bessoth	1999	Parallel lamination	20	50	1.5	0.07	60	Glass
[60]	Hadd	1997	T-mixer	35	9	1	0.014	35	Glass
[62]	Knight	1998	Focusing	10	10	50	0.5	500	Silicon-PDMS-glass
[64]	Walker	2004	Focusing	200-1000	150	1	0.15	200	PDMS-glass

n/a: not applicable

Figure 2.3: Serial lamination mixer: (a) join-split-join, (b) split-join [66], (c) split-split-join [69], (d) multiple intersecting microchannels [70].

Serial lamination micromixers

Similar to parallel lamination micromixers, serial lamination micromixers also enhance mixing through splitting and later joining the streams, Fig. 2.3a [66, 67, 68, 69]. The inlet streams are first joined horizontally and then in the next stage vertically. After m splitting and joining stages 2^m liquid layers can be laminated. The process leads to 4^{m-1} times improvement in mixing time. The mixers reported in [66, 67, 68] (Fig. 2.3b) were fabricated in silicon using wet etching in KOH or deep reactive ion etching (DRIE) technique. The same approach can be realized by lamination of multiple polymer layers [69], Fig. 2.3c.

The concept of serial lamination micromixer can also be applied to electrokinetic flows [70], Fig. 2.3d. Using electroosmosis flows between the multiple intersecting microchannels mixing is clearly enhanced. A similar design for a pressure-driven flow was reported in [71]. However, this design only works for a plug of the two mixed liquids.

Injection micromixers

The concept of injection mixer [72, 73, 74, 75, 76] is similar to parallel lamination mixer. Instead of splitting both inlet flows, this mixer type only splits the solute flow into many streams and injects them into the solvent flow. On top of one stream is an array

Table 2.2: Serial lamination micromixers.

Ref.	First author	Year	Number of stages	Channel width (μm)	Channel height (μm)	Typical velocity (mm/s)	Re	Pe	Materials
[66]	Branebjerg	1996	3	300	30	1-22	0.03-0.66	15-330	Silicon-glass
[67]	Schwesinger	1996	5-20	400	400	1.8	0.072	72	Silicon-glass
[68]	Gray	1999	6	200	100	n/r	n/r	n/r	Silicon-glass
[69]	Munson	2004	6	600	100	0.5	0.05	50	Mylar
[70]	He	2001	1	100	10	0.25	0.0025	25	Quartz
[71]	Melin	2004	16	50	50	2	0.1	14	Silicon-PDMS

n/r: not reported

Figure 2.4: Injection mixer: (a) two-dimensional model, (b) typical dimensionless concentration distribution of a micro plume ($Pe=1$).

of nozzles, which create a number of micro plumes of the solute. These plumes increase the contact surface and decreases the mixing path. Mixing efficiency can be improved significantly.

Figure 2.4a describes the two-dimensional model of the micro plume from a single circular nozzle of an injection mixer. The mixing chamber has a height of H , the flow rates of the solvent and the solute are $\dot{Q}_1$ and $\dot{Q}_2$, respectively. Assuming a uniform solvent velocity U and $\dot{Q}_1 \gg \dot{Q}_2$, the dimensionless concentration in a cylindrical coordinate $(\theta, r^* = r/R)$ is:

$$c^*(r^*, \theta) = \frac{K_0(Pe r^*/4)/Pe}{K_1(Pe/4) - K_0(Pe/4) \cos \theta} \{ \exp[Pe(r^* - 1)/4] \}^{\cos \theta}. \quad (2.11)$$

where K_0 and K_1 are the modified Bessel functions of the second kind. The Peclet number and the dimensionless concentration are defined as:

$$Pe = 2UR/D$$

and

$$c^* = \frac{c}{2\dot{Q}_2/(\pi H)},$$

respectively [77].

The injection micromixer reported in [72, 73] has 400 nozzles designed in a square array in a mixing chamber fabricated in silicon using DRIE. Larsen et al. [74] reported a

Figure 2.5: Planar designs for mixing with chaotic advection at high Reynolds numbers: (a) obstacles on wall [50], (b) obstacles in the channel [78, 79], (c) zigzag shaped channel [43].

similar concept with a different nozzle shape. In [75] and [76] capillary forces were utilized for generating microplumes. The mixers use a passive valve for releasing one of the two mixed fluids.

Micromixers based on chaotic advection

Besides diffusion, advection is another important form of mass transfer in flows at a low Reynolds number. However advection is often parallel to the main flow direction, and is not useful for the transversal mixing process. The so-called chaotic advection can improve mixing significantly. Generally, chaotic advection can be generated by special geometries in the mixing channel or induced by an external force. While the first type is a passive micromixer, the second type belongs to the active category and will be discussed later.

The design concept of micromixers based on chaotic advection is similar to their macroscopic counterparts, which are well investigated and summarized in [27]. The basic idea is the modification of the channel shape for splitting, stretching, folding and breaking of the flow. Following, micromixers for different ranges of Reynolds number are discussed individually. Although there are no fixed range for a particular design, we can consider the

ranges $Re > 100$ as high, $10 < Re < 100$ as intermediate, and $Re < 10$ as low.

Chaotic advection at high Reynolds number The simplest method to get chaotic advection is to insert obstacles in the mixing channel. A numerical investigation of obstacles at high Reynolds numbers was reported in [80]. The simulated mixing channel is $300\ \mu\text{m}$ in width, $100\ \mu\text{m}$ in depth and 1.2 mm to 2 mm in length, the diameter of the obstacle is $60\ \mu\text{m}$, Fig. 2.5b. Many arrangements of obstacles were investigated. The simulation assumed a Peclet number of 200. This work found that obstacles in a microchannel at low Reynolds numbers cannot generate eddies or recirculations. However, the results demonstrated that obstacles could improve mixing performance at high Reynolds numbers. Under this condition, the asymmetric arrangement of obstacles could alter the flow directions and forces fluids to merge and creates transversal mass transport. In [79], cylinders were used in a narrow channel to enhance mixing. The $50\ \mu\text{m} \times 100\ \mu\text{m} \times 100\ \mu\text{m}$ mixing chamber was fabricated using standard silicon technologies. Seven cylinders of $10\text{-}\mu\text{m}$ diameter were arranged in the mixing chamber. The micromixer worked with Reynolds numbers ranging from 200 to 2000 and a mixing time of $50\ \mu\text{s}$.

The next method to generate chaotic advection is using zigzag microchannels to produce recirculation around the turns at high Reynolds numbers. Based on a numerical investigation, Ménégaud et al. [43] discussed the periodic steps of the zigzag shape as the optimization parameter, Fig. 2.5c. The micromixers were fabricated using excimer laser on poly ethylene terephthalate (PET) substrate. The microchannel has a width of $100\ \mu\text{m}$, a depth of $48\ \mu\text{m}$ and a length of 2 mm. In the simulation, the Peclet number was fixed at 2600 and the Reynolds number ranged from 0.26 to 267. A critical Reynolds number of 80 was observed. Below this number the mixing process relies entirely on molecular diffusion. At higher Reynolds number, mixing was improved by the generated recirculations at the turns.

Chaotic advection at intermediate Reynolds numbers As mentioned above, micromixers based on chaotic advection can be derived from the macroscale designs using three-dimensionally twisted conducts [81]. Liu et al. [82] reported a three-dimensional serpentine mixing channel fabricated in silicon and glass. The channel was constructed as a series of C-shaped segments positioning in perpendicular planes, Fig. 2.6a. The micromixer consists of two inlet channels joined in a T-junction, a 7.5-mm-long straight chan-

Figure 2.6: Three-dimensional designs for mixing with chaotic advection at intermediate Reynolds numbers: (a) C-shape [82], (b) L-shape [83], (c) connected out-of-plane L-shapes [84], (d) twisted microchannel [44] (e,f,g) other designs of twisted channel [85]

nel, and a sequence of six mixing segments. The total mixing length was about 20 mm. An interesting observation from the micromixer is that the mixing process is faster at a higher Reynolds number. It shows that chaotic advection only occurs at relatively high Reynolds numbers ($Re=25-70$).

Vijayendran et al. [83] reported a three-dimensional serpentine mixing channel fabricated in PDMS. The channel was designed as a series of L-shaped segments in perpendicular planes, Fig. 2.6b. The channel has a width of 1 mm and a depth of $300\ \mu\text{m}$. The total length of the mixing channel is about 30 mm. The mixer was tested at the Reynolds numbers of 1, 5 and 20. The experimental results also indicated that better mixing was achieved at higher Reynolds numbers.

Another complex design on PDMS was reported by Chen and Meiners [84]. The mixing unit called by the authors "flow-folding topological structure" was formed by two connected out-of-plane L-shapes, Fig. 2.6c. This micromixer was also fabricated in PDMS. The microchannel has a width of $100\ \mu\text{m}$ and a depth of $70\ \mu\text{m}$. A single mixing unit measures about $400 \times 300\ \mu\text{m}$. With this design effective mixing can be achieved on short length scales with a purely laminar flow ($Re=0.1-2$).

A more complex three-dimensional micromixer was reported in [44]. This work fully utilized the theory in [27] to improve mixing in micro scale with complex and fine three-

dimensional channel shape. The channel rotates and separates the two fluids by partitioned walls and generates smaller blobs exponentially, Fig. 2.6d. This structure was made of PDMS on glass. Other designs of twisted microchannel were proposed in [85], but were not verified by fabricated prototypes. The channel has a width and height of $500\ \mu\text{m}$ and $300\ \mu\text{m}$, respectively. Mixing of a methanol and oxygen at different velocities (0.5 m/s to 2.5 m/s) was considered in the simulation, Fig. 2.6e-f.

A planar pulsed source-sink system can also cause chaotic advection in a mixing chamber [86]. The mixer was fabricated in silicon on a $1\ \text{cm}^2$ area. The mixing chamber measures $1500 \times 600\ \mu\text{m}$ with a height of $100\ \mu\text{m}$. For the details on this operation principle readers can refer to [87].

Chaotic advection at low Reynolds numbers Similar to macroscale mixers, rips (Fig. 2.7a) or grooves (Fig. 2.7b,c) on the channel wall can produce chaotic advection. Jonson et al. [88] were the first to investigate this phenomena. In this work, the grooves were ablated on the bottom wall using laser. This structure allows an electrokinetic flow to mix at a relatively slow velocity of $300\ \mu\text{m/s}$. This micromixers were fabricated by excimer laser ablation on a polycarbonate sheet (PC) covered of poly ethylene terephthalate glycol (PETG). The mixing channel was $72\ \mu\text{m}$ wide at the top, $28\ \mu\text{m}$ wide at the bottom, and $31\ \mu\text{m}$ deep. The width of an ablated groove was $14\ \mu\text{m}$, the center-to-center spacing between the grooves was $35\ \mu\text{m}$. The length of the region occupied by the wells from the T-junction was $178\ \mu\text{m}$.

Almost at the same time, Strook et al. investigated this effect and published their results in *Science* [89]. Two different groove patterns were considered (Fig. 2.7b, c). The so-called staggered herringbone mixer (Fig. 2.7b) can work well at a Reynolds number range from 1 to 100. This concept can be applied to electrokinetic flow by modifying the surface charge [90]. The effect of chaotic advection with the ripped channel was numerically investigated by Wang et al. [78]. The length, width and depth of the channels were 5 mm, $200\ \mu\text{m}$, and $100\ \mu\text{m}$, respectively. The mean velocity ranges from $100\ \mu\text{m/s}$ to 50 mm/s. The grooves were also ablated on the PDMS substrate by laser [48]. Electrokinetic mixing [91] with only patterned surface modification can also enhance mixing, Fig. 2.7d,e,f. With a field strength of 70-555 V/cm along the 1.8-mm-long microchannel, mixing efficiencies can be improved by 22-68% at Pe 190-1500. The concept of surface modification can be found in

Figure 2.7: Modification of mixing channel for chaotic advection at low Reynolds numbers: (a) slanted ribs, (b) slanted grooves [89, 90], (c) staggered-herringbone grooves [89, 90], (d-f) patterns for surface modification in a micromixer with electrokinetic flows [91].

[92].

The design of [89] was improved with embedded barriers parallel to the flow direction [93]. The mixing channel of this design is $240\ \mu\text{m}$ in width, $60\ \mu\text{m}$ in depth and $21\ \text{mm}$ in length. The barriers have a cross section of $40\ \mu\text{m} \times 30\ \mu\text{m}$. This embedded barrier changes the original elliptic mixing pattern [89] to a hyperbolic pattern [93].

A miniaturized version of a conventional mixer with helical elements was reported in [94]. This conventional static mixers with helical elements is also called the Kenics static mixer [95]. The concept changed the three-dimensional inner surface of a cylindrical mixing channel. Two designs were reported for this mixer type. The first design was formed by four mixing elements, which was made of 24 rectangular bars placed at 45° . The four mixing elements were arranged at 45° in the channel. The second design consists of right-handed and left-handed helical elements containing 6 small-helix structures. The micromixer was fabricated by stereo microphotography, which builds up the complex structure layer by layer.

Droplet micromixers

Another solution for reducing the mixing path is to form droplets of the mixed liquids. The movement of a droplet causes an internal flow field and makes mixing inside the droplet possible. In general, droplets can be generated and transported individually using pressure [96] or capillary effects such as thermocapillary [41] and electrowetting [97]. Furthermore,

Table 2.4: Chaotic advection micromixers.

Ref.	First author	Year	Type	Channel width (μm)	Channel height (μm)	Typical velocity (mm/s)	Re	Pe	Materials
[80]	Wang	2002	cyl. obstacles	300	100	0.17	0.25	51	n/a
[79]	Lin	2003	cyl. obstacles	10	100	20	0.2	200	Silicon-glass
[43]	Mengeaud	2002	zigzag shaped	100	48	1.3-40	0.26-267	130-4000	Mylar
[82]	Liu	2000	3D serpentine	300	150	30-350	6-70	9000-10 ⁴	Silicon-glass
[83]	Vijayendran	2003	3Dserpentine	1000	300	2-40	1-20	2000-4 $\times$ 10 ⁴	PDMS
[84]	Chen	2004	3D serpentine	100	70	1-20	0.1-2	10-200	PDMS
[44]	Park	2004	3D serpentine	100	50	n/r	1-50	0.015-0.7	PDMS
[85]	Jen	2003	3D serpentine	500	300	2000	48	0.36	n/a
[86]	Evans	1997	Source-sink	1500 $\times$ 600	100	n/r	n/r	n/r	Silicon-glass
[88]	Johnson	2002	Patterned wall	72	31	0.6	0.024	15	PC-PETG
[89, 90]	Stroock	2002	Patterned wall	200	70	15	0.01	3000	PDMS
[78]	Wang	2003	Patterned wall	200	100	0.1-50	0.0013-6.65	20-10 ⁴	PDMS
[91]	Biddiss	2004	Patterned wall	200	8	0.01-0.09	0.08-0.7	190-1500	PDMS
[93]	Kim	2004	Patterned wall	240	60	11.6	0.5	2784	PDMS

n/r: not reported; n/a: not applicable

Table 2.5: Droplet micromixers.

Ref.	First author	Year	Transport type	Droplet size (nL)	Channel width (μm)	Channel height (μm)	Materials
[96]	Hosokawa	1999	Pressure driven	10	100	150	PDMS/PMMA
[97]	Paik	2003	Electrowetting	1600	2480	600-1000	Glass
[33]	Song	2003	Multiple phases	75-150	20-100	n/r	PDMS

n/r: not reported

droplets can be generated due to the large difference of surface forces in a small channel with multiple immiscible phases such as oil/water or water/gas [33].

The earliest droplet micromixer was fabricated in PDMS and covered by a PMMA sheet [96]. The concept utilized a hydrophobic microcapillary vent, which joints the two initial droplets.

By simplifying the mass transport equation and introducing an effective dispersion coefficient for a rectangular channel, Handique and Burns reported an analytical model for droplet mixing actuated by thermocapillary [41].

The droplet can also be transported by electrowetting [97]. Different mixing schemes can be used with this concept. Droplets can be merged and split repeatedly to generate the mixing pattern. The merged droplet can be transported around using electrowetting.

The other droplet micromixer design used flow instability between two immiscible liquids [33, 98]. Using a carrier liquid such as oil, droplets of the aqueous samples are formed. While moving through the microchannel, the shear force between the carrier liquid and the sample accelerated the mixing process in the droplet.

2.2.2 Active micromixers

Pressure field disturbance

Pressure field disturbance was used in one of the earliest active micromixers. Deshmukh et al. [99] reported a T-mixer with pressure disturbance, Fig. 2.8a. The mixer is integrated in a microfluidic system, which is fabricated in silicon using DRIE. An integrated planar micropump drives and stops the flow in the mixing channel to divide the mixed liquids into serial segments and make the mixing process independent of convection,

Figure 2.8: Active micromixers: (a) serial segmentation, (b) pressure disturbance along mixing channel, (c) integrated micro stirrer in the mixing channel, (d) electrohydrodynamic disturbance, (e) dielectrophoretic disturbance, (f) electrokinetic disturbance in a mixing chamber, (g) electrokinetic disturbance in a mixing channel.

Fig. 2.8a. The performance of this micromixer was later discussed in [42]. The pressure disturbance can also be generated by an external micropump [100].

Another alternative method to pressure disturbance is the generation of pulsing velocity [101, 102], Fig. 2.8b. Glasgow et al. [101] demonstrated a simple T-mixer and its simulation with a pulsed side flow at a small Reynolds number of 0.3. The paper did not elaborate further on the generation of the pulsed flow. In [102], the pressure disturbance was achieved by introducing a computer controlled source-sink system. This design is partly similar to [86]. The performance of the mixing process is related to the pulse frequency and the number of mixing units. A further modelling work on pressure disturbance was reported in [103]. However, the analysis focused only on the mixing pattern in the chamber.

Suzuki and Ho [104] reported a micromixer with integrated conductors. The electrical conductors generate a magnetic field, which in turn moves magnetic beads of $1\text{ }\mu\text{m}$ to $10\text{ }\mu\text{m}$ in diameters. The disturbance caused by the magnetic beads improves mixing significantly. Disturbance can also be generated by an integrated magnetic microstirrer as reported in [105], Fig. 2.8c. The micromachined microstirrer is placed at the interface between two liquids in a T-mixer. An external magnetic field drives the stirrer at a speed between 100 rpm and 600 rpm.

Electrohydrodynamic disturbance

The structure of the micromixer with electrohydrodynamic disturbance [106] is similar to the concept reported in [102]. Instead of pressure sources, electrodes are placed along the mixing channel, Fig. 2.8d. The mixing channel is 30 mm long, $250\text{ }\mu\text{m}$ wide and $250\text{ }\mu\text{m}$ deep. A series of titanium wires are placed in the direction perpendicular to the mixing channel. By changing the voltage and frequency on the electrodes, good mixing was achieved after less than 0.1 sec at a low Reynolds number of 0.02.

Dielectrophoretic disturbance

Dielectrophoresis (DEP) is the polarization of a particle relatively to its surrounding medium in a non-uniform electrical field. This effect causes the particle to move to and from an electrode. A dielectrophoretic micromixer was reported in [107, 108]. Chaotic

advection was generated by embedded particles with combination of electrical actuation and local channel variation, Fig. 2.8e.

Electrokinetic disturbance

As mentioned above, electrokinetic flow can be used to transport liquid in micromixers as an alternative to pressure driven flow. Jacobson et al. [61] reported electrokinetically driven mixing in a conventional T-mixer. Lettieri et al. proposed the use of electrokinetic effect to disturb the pressure driven flow in a micromixer [109]. In another case [110], oscillating electroosmotic flow in a mixing chamber is caused by an AC-voltage. The pressure driven flow becomes instable in a mixing chamber (Fig. 2.8f) or in a mixing channel (Fig. 2.8g).

Tang et al. also utilized an electrokinetic flow to improve mixing [111]. Similar to the previous pressure-driven approach [99], switching on or off the voltage supplied to the flow generates fluid segments in the mixing channel. This flow modulation scheme was capable of injecting reproducible and stable fluid segments into microchannels at a frequency between 0.01 Hz and 1 Hz.

Magneto hydrodynamic disturbance

Magneto hydrodynamic effect [112] has been used in micromixer. In the presence of an external magnetic field applied DC-voltages on the electrodes generate Lorentz forces, which in turn induces mixing movement in the chamber. The Lorentz force can roll and fold the liquids in a mixing chamber. This concepts only works with an electrolyte solution. The mixer of [112] was fabricated from co-fired ceramic tapes. The electrodes are printed with a gold paste.

Acoustic disturbance

Acoustic actuators were used to stir fluids in micromixers. The proof of concept for acoustic mixing was reported by Moroney et al. [113] with a flexible-plate-wave (FPW) device. Zhu and Kim [114] gave an analysis of focused acoustic wave model in a mixing chamber. They demonstrated an acoustic micromixer fabricated in silicon. The mixing

chamber measures $1\text{ mm} \times 1\text{ mm} \times 10\text{ }\mu\text{m}$. A zinc oxide membrane is located at the bottom of the mixing chamber. The vibration can be controlled by changing the frequency and the voltage of the input signal. The concept of acoustically induced flow, or acoustic streaming, was also used as an active mixing scheme [115]. Focused acoustic streaming with different electrode patterns was used for mixing [116]. Besides the integrated design, stirring at high frequency can also be realized by an external pump [117].

Ultrasonic mixing may have problems in applications for biological and chemical analysis. The reason is the temperature rise caused by acoustic energy. Many biological fluids are highly sensitive to temperature. Furthermore, ultrasonic waves around 50 KHz are harmful to biological samples because of the possible cavitations. The acoustic micromixer reported in [118] uses loosely-focused acoustic waves to generate stirring movements. The wave is generated by a piezoelectric zinc oxide thin film. The actuator was driven by sinusoidal wave with frequencies corresponding to the thickness-mode resonance (e.g. 240 MHz and 480 MHz) of the piezoelectric film. The mixer operated without any significant temperature increase and could be used for temperature-sensitive fluid. Further acoustic devices for mixing water and ethanol as well as water and uranine was reported in [119, 120].

Acoustic streaming induced around an air bubble was used in [121, 122] for mixing. In this mixer, air pockets with $500\text{ }\mu\text{m}$ diameter and $500\text{ }\mu\text{m}$ in depth were used for trapping air bubbles. Acoustic streaming was induced by the field generated by an integrated PZT actuator. Acoustic streaming was also used in [123] to disturb the flow in a conventional Y-mixer. While the channel was made of PDMS, the acoustic actuator was integrated into the cover quartz wafer. A $8\text{-}\mu\text{m}$ thick zinc oxide layer with gold electrodes works as the actuator.

Thermal disturbance

Since the diffusion coefficient depends highly on temperature, thermal energy also can be used to enhance mixing [124, 125]. Mao et al. [124] generated a linear temperature gradient across a number of parallel channels in order to investigate the temperature dependence of fluorescent dyes. This approach can be possibly used for mixing in microscale. The other design [125] utilized a thermal bubble to generate disturbance in a mixing channel.

Table 2.6: Active micromixers.

Ref.	First author	Year	Disturbance	Channel width (μm)	Channel height (μm)	Typical velocity (mm/s)	Frequency (Hz)	Re	Pe	St	Materials
[42]	Deshmukh	2001	Pressure	400	78	0.09	1	0.01	36	4.4	Silicon-glass
[100]	Fuji	2003	Pressure	150	150	0.9	100	0.13	133	17	PDMS
[101]	Glasgow	2003	Pressure	200	120	2	0.3	0.3	400	0.03	n/a
[103]	Okkels	2004	Pressure	200	26	1.6	0.85	0.04	321	0.11	PDMS
[104]	Suzuki	2002	Pressure	160	35	0.3	0.02	0.05	48	4	Silicon-glass
[105]	Lu	2002	Pressure	750	70	0.14	5	0.01	105	n/a	PDMS-glass
[106]	ElMoutar	2003	Electrohydrodynamic	250	250	4.2	0.5	0.02	1050	0.03	n/a
[107]	Deval	2002	Dielectrophoretic	50	25	0.5	1	0.02	25	0.1	Si-SU8-glass
[108]	Lee	2001	Electrokinetic	200	25	0.5	1	0.01	100	0.4	n/a
[110]	Oddy	2001	Electrokinetic	1000	300	0.5	10	0.15	1050	20	PDMS-glass
[111]	Tang	2002	Electrokinetic	500	35	1	0.17	0.04	509	0.09	PDMS-glass
[112]	Bau	2001	Magneto hydrodynamic	4700	1000	n/r	n/r	n/r	n/r	n/r	Ceramic
[113]	Moroney	1991	Acoustic	1000	400	0.5	10	0.15	1050	20	Si-glass
[115]	Rife	2000	Acoustic	1600	1600	1	n/r	1.6	1600	n/r	n/r
[118]	Yasuda	2000	Acoustic	2000	2000	6.4	n/r	12.8	12800	n/r	Si-glass
[120]	Yang	2001	Acoustic	6000	60	0.5	n/r	0.03	30	n/r	Si-glass
[121]	Liu	2002	Acoustic	15000	300	5	n/r	1.5	1500	n/r	Si-glass
[123]	Yaralioglu	2004	Acoustic	300	50	1	n/r	0.86	300	n/r	PDMS-Quartz

n/r: not reported; n/a: not applicable

2.2.3 Discussions

Operation conditions

The operation conditions of micromixers can be determined by the characteristic dimensionless numbers such as Reynolds number Re and Peclet number Pe . From the definitions (2.8) and (2.9), the relation between Pe and Re can be derived as:

$$\frac{Pe}{Re} = \frac{L}{D_h} \frac{\nu}{D} \quad (2.12)$$

The hydraulic diameter D_h and the mixing path L are usually on the same order, therefore we can assume $L/D_h \approx 1$. The kinematic viscosity and the diffusion coefficient of liquids are on the order $\nu = 10^{-6} \text{ m}^2/\text{s}$ and $D = 10^{-9} \text{ m}^2/\text{s}$, respectively. Thus, based on (2.12) the relation between Peclet number and Reynolds number can be estimated for liquids as $Pe \approx 1000Re$. On a $Pr-Re$ diagram, the relation $Pe=1000Re$ represents a straight line. Operation points of micromixers for liquids are expected to be around this line. Similarly, for gases with a typical kinematic viscosity and a diffusion coefficients of $\nu = 10^{-5} \text{ m}^2/\text{s}$ and $D = 10^{-5} \text{ m}^2/\text{s}$, the operation point can be expected around the line of $Pe=Re$. Figure 2.9 depicts the operation points of the mixers reviewed in this chapter with the two characteristic lines for gas and liquid. The Reynolds and Peclet numbers are calculated based on the typical values of kinematic viscosity and diffusion coefficient mentioned above, if no further data is given in the particular work.

The data points in Fig. 2.9 shows clearly the two operation areas for liquids and gases. Many points are lying above the $Pe = 1000Re$ line, because the ratio between the mixing path L and the hydraulic diameter D_h is more than unity in most cases. In a planar microfluidic system the channel width, which usually represents the mixing path, is much larger than the channel height, which is usually close to value of the hydraulic diameter. Figure 2.10 depicts the most important characteristics of a $Pe-Re$ diagram.

Figure 2.11 depicts the typical Strouhal numbers of active micromixers with pressure disturbance and electrokinetic disturbance. We can clearly observe that a higher Reynolds number requires a higher Strouhal number. A fast flow has a short residence time in the mixing channel, thus a shorter time period or a higher disturbance frequency is needed for full mixing. Since the disturbance frequency depends on the dynamics of the external

Figure 2.9: Typical operation ranges of micromixers. The Reynolds number represents the flow range in the mixing channel, while the Peclet number represents the ratio between convection and diffusion. The common flow range in microfluidic devices is $Re < 1$. The data points were determined based on reported geometry data and velocity (flow rate) data. If the kinematic viscosity ν and the diffusion coefficient D are unknown, characteristic values for liquids of $\nu = 1 \times 10^{-6} \text{ m}^2/\text{s}$ and $D = 1 \times 10^{-9} \text{ m}^2/\text{s}$ are assumed. The two characteristic lines $Pe = 1000Re$ and $Pe = Re$ for liquids and gases, respectively, are explained in the text.

Figure 2.10: The Pe - Re diagram. Passive micromixers either work at low Reynolds number and low Peclet numbers (bottom left corner) or at high Reynolds number in the transition regime to turbulence (top right corner). Operation points of passive mixers based on chaotic advection and active mixers can be distributed around the characteristic lines for liquids and gases for a wide range of Reynolds number. Passive lamination micromixers with multiple streams have typically small Peclet numbers ($Pe < 100$). In the range of ($Pe < 1000$), the mixer can be considered as diffusion based.

actuator, the Reynolds number or the flow rate of the mixer should be designed to match a given disturbance frequency.

Fabrication techniques

A variety of fabrication techniques have been used for making micromixers. The different techniques can be categorized as silicon micromachining and polymeric micromachining.

Most of the early micromixers were made of silicon. The mixing channels were either wet etched with KOH [35, 55, 66, 67, 72, 82] or dry etched using DRIE (deep reactive ion etching) [47, 49, 52, 57, 68, 76, 79, 99]. A glass cover is anodically bonded on top of the channel offering both sealing and optical access. Passive micromixers can be made entirely of glass [59, 60, 61]. In some applications such as mixing of electrokinetic flows, silicon can not be used because of its electrically conducting properties. Most active micromixers with integrated actuators are fabricated in silicon because of the established technologies [104, 113, 117, 120] such as sputtering of metals and piezoelectric materials.

Besides the advantages of an established technology, silicon-based micromixers are relatively expensive because of the large surface needed for microchannels and the required clean room facilities. Furthermore, silicon devices are chemically and biochemically not always compatible. Polymeric micromachining offers a lower fabrication cost and a faster prototyping cycle. A simple approach established by the Whitesides's group [126] at Harvard University has been repeated recently by many other groups [48, 64, 89, 90, 110, 111]. This low-cost approach uses lithography mask printed from high-resolution laser printer. The mask is then used for the subsequent photolithography of the thick-film negative resist SU-8 on a silicon wafer. The SU-8/silicon wafer works as a mold for an elastomer such as polydimethylsiloxane (PDMS). After a surface treatment in oxygen plasma, the PDMS part with the microchannels can be covered by a glass plate, which provides both optical transparency and mechanical rigidity for the device. Several PDMS layers can be fabricated in the same way and bonded directly to form a complex three-dimensional structure [83, 84, 44, 93]. For direct bonding, methanol was used for self-alignment between the PDMS-layers. Because of its sealing property, PDMS can also be used as the adhesion layer between glass and silicon [62].

Figure 2.11: The St-Re diagram. Strouhal number represents the ratio between residence time and the time period of the disturbance. The data indicate that a higher Reynolds number (high flow velocity) requires a larger Strouhal number (a higher disturbance frequency).

The thick-film resist SU-8 can be used directly for making micromixers. SU-8 microchannels were formed on a silicon or glass substrate [53, 107]. SU-8 has the advantage of simple micromachining. Moveable structures such as microvalves [127] and microgrippers [128] have been fabricated with the so-called polymeric surface micromachining. This approach proves the feasibility of making a complex microfluidic system with moveable structures in SU-8.

Mixing channels were also fabricated by hot embossing with a hard template, which is micromachined in silicon [88]. This approach is limited to a two-dimensional channel structure but promises a simple way for mass production. Fast prototyping can be achieved with laser micromachining of thin polymer and adhesive sheets [45, 69]. However, the resolution of this approach is limited by the wavelength of the laser.

Characterization techniques

Despite of the recent numerous works on micromixers, characterization of micromixers still remains a challenge. The quantification of the extent of mixing is important for evaluation of performance as well as design optimization of micromixers.

The common quantification technique is using dilution of a tracer dye to determine the extent of mixing. For a low-noise measurement, fluorescent dye and a corresponding microscope with a filter set is required. The intensity image can then be recorded and evaluated. Since the concentration of the dye is proportional to the intensity of the recorded image, the uniformity of concentration image can be quantified by determining the standard deviation of the pixel intensity values [82, 89]. In some cases, if the standard deviation of intensity values can not resolve the differences between regions in the image, spatial probability density functions (PDF) of intensities integrated over a finite regions can be used to quantify mixing [110]. Furthermore, two-dimensional power spectrum of the intensity image can also be considered as another quantification method [110, 102].

The above techniques are statistical methods, which depends on the orientation of the mixed fluids relative to the imaging direction. If the imaging direction is perpendicular to the fluid layers as in the case of [65], the two layers, even at the channel's entrance, appear to be completely mixed. In such cases, an imaging system with a confocal microscope is required for the three-dimensional spacial distribution of the concentration field [31, 62]

Another quantification method measuring the fluorescent product of a chemical reaction [35]. The intensity of the product is a direct measure for the extent of mixing. Typically, this process is an acid-base reaction with a dye having a fluorescence quantum yield that is pH dependent. Recently, Munson and Yager [69] reported a new concept for the quantification of mixing. The method relies on the increase of intensity of fluorescein at basic pH. In this method, both liquids are diluted with fluorescein. They only have different buffers with different pH values. The increase in fluorescence in the initially acidic solution overwhelms the small decrease in fluorescent of the other solution. The total fluorescence increases by a factor of 2 and can work as a measure for the extent of mixing [69].

Applications of micromixers

Micromixers are widely used in chemical, biological, and medical analysis fields. Almost every chemical assay requires mixing of reagents with a sample.

The basic T-mixer was used in [35] for measurement of analyte concentrations of a continuous flow. The concentration of a target analyte is measured with the fluorescence intensity of the region where the analyte and a fluorescent indicator have interdiffused [35, 31]. The micromixer reported in [65] was used for the study of rapid chemical reactions in solution with stopped-flow time resolved Fourier transform infrared spectroscopy (TR-FTIR). Wu et al. [45] used a Y-mixer for investigating nonlinear diffusive behavior of a fluorescein. Micromixers can be used as sensors in environmental monitoring such as detection of ammonia in aqueous solutions [52]. The fast mixing time in a micromixer benefits time-resolved measurement of reaction kinetics using nuclear magnetic resonance (NMR) [58]. Fluri et al. [59] combines capillary electrophoresis (CE) separation with a T-mixer as a postcolumn reactor. An electrokinetically driven T-mixer was used in [60] for performing enzyme assays. The short mixing length of a cross-mixer with hydrodynamic focusing makes fast infection of a cell with virus possible [64]. Fast mixing with a micromixer was used for freeze-quenching technique, which is useful for trapping metastable intermediates populated during fast chemical or biochemical reactions [79]. In [83] micromixers were used for sample preparation of a surface-based biosensor.

Besides sensing and analysis applications as discussed above, Micromixers was used as a tool for dispersing immiscible liquids and forming micro droplets [56]. Furthermore,

micromixers work as a separator for particles based on their different diffusion coefficients [12, 129] or as a generator of concentration gradients [130, 131, 36, 132, 133].

2.3 Remarks on previous works

Many works have been done in theory, device development and characterization of micromixers. However, there are still some problems that need to be solved. In previous works, the common approach is ignoring the diffusion effects along the flow direction [36, 35] or assuming full mixing at a fixed length [37]. For cases with small Peclet number, these assumptions are not correct. In such a case diffusion effect is significant, and cannot be ignored. Parallel mixing is often used in micromixer designs, but there are no model for parallel mixing. Furthermore, the mixing ratio is often assumed as 1:1 in previous works. In many applications, other mixing ratios are needed. All previous works assume the same viscosity in the mixing streams. This is not always true in many applications. Furthermore, as mentioned before, the micro effects such as molecular interactions are often ignored. Many works such as [32, 35, 37, 41] only consider linear models.

With potentials in analytical chemistry and bio assays, new requirements are proposed for the design and fabrication of micromixer. The requirements include low cost, relative simple fabrication technique and process, low reagents/sample consumption and stable operation. Micromixers should be easy to be integrated in a microfluidic system. Thus, active micromixers are not attractive for their complex designs. Micromixers working at high Reynolds number also have drawbacks. High Reynolds number means high velocity and high reagents/sample consumption. Furthermore, polymer materials are preferable for their low cost and biocompatibility.

Chapter 3

Modelling of transport effects in microchannels

In this chapter, a number of analytical models are formulated to discuss the transport effects in the basic Y-mixer. First of all, a two-dimensional convective-diffusive model of diluted mixing with a 1:1 ratio is established and solved analytically. This model is then extended to mixing with an arbitrary mixing ratio as well as with multiple mixing streams. Furthermore, Taylor dispersion is considered in the later model. Besides mixing of streams with the same viscosity, mixing between streams with different viscosities are also considered. Hydrodynamic focusing helps to decrease the mixing path and to enhance the mixing effect. An analytical model for hydraulic focusing is also discussed in this chapter. Because of the difficulty of an analytical solution for the case of mixing between streams with different viscosities, numerical simulation is used. Finally, non-linear effects of mixing in a microchannel are discussed.

Due to the small dimension and consequently the low Reynolds number in microfluidics, the following models only consider the flow under small Reynolds number at isothermal conditions.

3.1 Mixing with the same viscosity

The model is a straight microchannel with a rectangular cross section, Fig. 3.1. The channel has two inlets for the solute and the solvent and one outlet for the mixed solution.

Figure 3.1: The physical model and its parameters.

The width, height and length of the channel are W , H and L , respectively.

3.1.1 Governing equations

Generally, the fluid in the mixing channel can be considered as incompressible and Newtonian. Under the assumption of a constant temperature, the behavior of the fluid flow in the mixing channel can be described by the three governing equations:

- the continuity equation

$$\frac{\partial \rho}{\partial t} \nabla \vec{v} = 0, \quad (3.1)$$

- the momentum equation

$$\frac{\partial \vec{v}}{\partial t} + (\vec{v} \cdot \nabla) \vec{v} = \vec{F} - \frac{1}{\rho} \nabla p + \nu \nabla^2 \vec{v}, \quad (3.2)$$

- and the transport equation

$$\frac{\partial c_i}{\partial t} + \vec{v} \cdot \nabla c_i = D_i \nabla^2 c_i + r_i. \quad (3.3)$$

In the above equations, ρ is the density of the fluid, t is the time, $\vec{v}$ is the velocity vector, p is the pressure, $\vec{F}$ is the external force, ν is the kinetic viscosity of the flow, c_i is the concentration of specie i , D_i is the diffusion coefficient of specie i , and r_i is the reaction rate specie i .

3.1.2 Solution of the velocity distribution

In microchannels, the flow is usually laminar. Often, the Reynolds number Re is on the order of 1 or less. The viscous force is dominant. Thus, the flow is fully developed

and becomes steady state right after the entrance. When the channel length is much larger than the channel width as in the case of the micromixer considered here, entrance effects are also negligible [1]. Ignoring the entrance effects, the velocity in the channel can be considered as fully-developed along the channel length. Thus the velocity components v and w can be neglected. Under this assumption, equation (3.2) automatically satisfies (3.1) and can be simplified as:

$$\frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2} = \frac{1}{\nu} \frac{dp}{dx} \quad (3.4)$$

With the boundary conditions:

$$u|_{y=0,z} = u|_{y=W,z} = u|_{y,z=\pm H/2} = 0, \quad (3.5)$$

the analytical solution of (3.4) can be obtained by separating the variables. Using dimensionless variables $x^* = x/W$, $y^* = y/W$, and $z^* = z/W$, the dimensionless velocity is derived as:

$$u^* = P' \left[\frac{z^{*2} - h^2/4}{2} + \sum_{n=1}^{\infty} \cos \theta z^* (A_n \cosh \theta y^* + B_n \sinh \theta y^*) \right], \quad (3.6)$$

where $P' = \frac{W}{u_0 \nu} \frac{dp}{dx^*}$, u_0 is the reference velocity, $h = 2H/W$, and $\theta = (2n-1)\pi/h$. The coefficients A_n and B_n are determined by Fourier analysis:

$$A_n = (-1)^{n+1} \frac{4h^2}{(2n-1)^3 \pi^3} \quad (3.7)$$

$$B_n = \frac{A_n (1 - \cosh \theta)}{\sinh \theta} \quad (3.8)$$

Figure 3.2 shows two typical velocity distributions of this model. For a flat channel $h \ll 1$ the velocity distribution in $x^* - y^*$ plan is almost uniform as in the case of a two-dimensional Hele-Shaw flow. In reality, the channel height h is often much smaller than the channel width. Thus, the mixing model can assume a uniform velocity profile in the $x^* - y^*$ -plane. For velocity distribution along the z^* -axis, the well-known parallel-plate model can then be used. Solving the one-dimensional Navier-Stokes equation leads to the typical parabolic velocity profile along z^* -axis.

Figure 3.2: The typical dimensionless velocity distributions: (a) $h=0.2$ (b) $h=0.1$

3.1.3 Solution of the concentration distribution

The concentration distribution is obtained by solving the transport equation (3.3). This equation can not be solved analytically in the three-dimensional form even with the assumption of a fully developed flow. A simplification of the model is therefor needed. From the observations discussed in the previous section, the velocity profile in $x^* - y^*$ plan is almost uniform, so a constant velocity can be assumed for the mixing model. Furthermore, species transport of steady side-by-side flow in flat channels is not affected by the velocity distribution along the channel height. The velocity for the convective transport can be approximated by the averaged velocity in the microchannel [36]. From the experimental point of view, the collected fluorescence signal under normal microscope is averaged over the channel depth. Under these assumptions, the transport equation reduces to a two-dimensional form. Following, several mixing models are discussed: mixing with 1:1 ratio, mixing with an arbitrary ratio without and with Taylor dispersion.

Mixing with 1:1 ratio

The two-dimensional model of a micromixer with two inlet streams is depicted in Fig. 3.3. The mixer is a long channel with a width of W and two inlet streams. One stream is the solute with a concentration of c_0 , the other stream is the solvent with a concentration of $c = 0$. We consider the mixing effect of a binary system with a solute flow and a solvent flow. There is no reaction between the solute and the solvent. The flow in the channel is

Figure 3.3: Two-dimensional model of a micromixer: (a) the actual model, (b) the dimensionless model.

assumed to have a constant velocity of U . The boundary conditions are depicted in Fig. 3.3a. The Equation (3.3) becomes:

$$D \left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} \right) = U \frac{\partial c}{\partial x} \quad (3.9)$$

where D is the diffusion coefficient of the solute. By introducing the dimensionless coordinate system $x^* = x/W$, $y^* = y/W$, the normalized concentration $c^* = (c - c_0/2)/c_0$ and the Peclet-number :

$$\text{Pe} = \frac{UW}{D}, \quad (3.10)$$

Equation (3.9) can be formulated in the dimensionless form as:

$$\left(\frac{\partial^2 c^*}{\partial x^{*2}} + \frac{\partial^2 c^*}{\partial y^{*2}} \right) = \text{Pe} \frac{\partial c^*}{\partial x^*}. \quad (3.11)$$

The dimensionless model with its boundary conditions is depicted in Fig. 3.3b. The boundary conditions for (3.9) are:

$$\begin{aligned} c^* \Big|_{(x^*=0, 0 < y^* < 1/2)} &= 1/2 \\ c^* \Big|_{(x^*=0, -1/2 < y^* < 0)} &= -1/2 \\ c^* \Big|_{(x^*=\infty)} &= 0. \end{aligned} \quad (3.12)$$

Figure 3.4: Normalized concentration distribution in the channel with 1:1 mixing ratio: (a) $Pe=100$ two inlets, (b) $Pe=250$ two inlets, (c) $Pe=100$ multiple inlets, (d) $Pe=250$ multiple inlets.

The channel walls are impermeable. Therefore, the zero flux boundary condition can be applied for the channel walls:

$$\left. \frac{\partial c^*}{\partial y^*} \right|_{y=\pm 1/2} = 0 \quad (3.13)$$

Separating the variables in (3.9) and applying corresponding boundary conditions (3.12, 3.13) result in the dimensionless concentration:

$$c^*(x^*, y^*) = \frac{1}{\pi} \sum_{n=1}^{\infty} \exp \left[\frac{Pe - \sqrt{Pe^2 + 4(2n-1)^2 \pi^2}}{2} x^* \right] \times \sin [\pi(2n-1)y^*] \frac{1 - \cos[\pi(2n-1)]}{2n-1}. \quad (3.14)$$

$n = 1, 2, 3 \dots$

Figure 3.4 represents graphically some typical results of the concentration distribution.

Figure 3.5: Two-dimensional model of arbitrary ratio mixing: (a) the actual model, (b) the dimensionless model.

Mixing with an arbitrary ratio

In the previous section, the mixing ratio of solute and solvent is assumed to be 1:1. In practical applications, other ratios of solute and solvent are often required. Following, r is defined as the mass fraction of solute in the final mixed solution. The boundary conditions are depicted in Fig. 3.5a. The mixing ratio between the solute and the solvent is then $(r : 1 - r)$. Using the similar approach of the previous section with a dimensionless concentration $c^* = (c - rc_0)/c_0$, the dimensionless model and its corresponding boundary conditions are depicted in Fig. 3.5b. The governing equation for this model is:

$$\text{Pe} \frac{\partial c^*}{\partial x^*} = \frac{\partial^2 c^*}{\partial x^{*2}} + \frac{\partial^2 c^*}{\partial y^{*2}}. \quad (3.15)$$

with the boundary conditions for the inlets:

$$f(y^*) = \begin{cases} c^* \big|_{(x^*=0, 0 \leq y^* < r)} = 1 - r \\ c^* \big|_{(x^*=0, y^*=r)} = 0 \\ c^* \big|_{(x^*=0, r < y^* \leq 1)} = -r \end{cases}, \quad (3.16)$$

the boundary conditions for the outlet:

$$\frac{\partial c^*}{\partial x^*} \big|_{(x^*=\infty, 0 \leq y^* \leq 1)} = 0, \quad (3.17)$$

and the boundary conditions for the walls:

$$\left. \frac{\partial c^*}{\partial y^*} \right|_{y^*=0,1} = 0. \quad (3.18)$$

Solving the equation with its corresponding boundary conditions, the solution of the concentration distribution has the dimensionless form:

$$c^*(x^*, y^*) = \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{\sin nr\pi}{n} \cos(n\pi y^*) \times \exp \left(-\frac{2n^2\pi^2}{\text{Pe} + \sqrt{\text{Pe}^2 + 4n^2\pi^2}} x^* \right) \quad (3.19)$$

$n = 1, 2, 3, \dots$

Obviously, it is a more general solution for diluted mixing of a binary system. For example, when $r=0$ or $r=1$, the right side of Equation (3.19) becomes zero, because there is no mixing effect for a single phase. When $r = 1/2$, the conditions are identical as the case of 1:1 ratio of the previous section. Thus, the solution of the previous section can be considered as a special case of (3.19). Figure 3.6 shows some typical results of the concentration distribution.

Arbitrary ratio mixing with Taylor dispersion

As already mentioned in section 3.1.2, the velocity in z^* -axis can be assumed as parabolic. Because of the non-uniformity of the velocity along the channel height, the mass transport is consequently affected. The so-called effect of Taylor dispersion occurs in the $y^* - z^*$ -plane. According to [40], the dispersion in $y^* - z^*$ -plane can be considered in a model with an effective diffusion coefficient in x direction:

$$D_x = D + \frac{1}{210} \frac{U^2 H^2}{D} \quad (3.20)$$

where U is the assumed uniform velocity along x -axis. The diffusion coefficient D_y along y -axis is equal to D . With this new assumptions, we consider here the mixing effect of a binary system with a solute flow and a solvent flow. There is no reaction between the solute and the solvent. Equation (3.3) can be simplified to the two-dimensional form:

$$U \frac{\partial c}{\partial x} = D_x \frac{\partial^2 c}{\partial x^2} + D_y \frac{\partial^2 c}{\partial y^2} \quad (3.21)$$

Figure 3.6: Typical results of the analytical model for $Pe = 50$: (a) two streams, mixing ratio 1:1, (b) two streams, mixing ratio 4:1, (c) multiple streams, mixing ratio 1:1, (d) multiple streams, mixing ratio 1:4.

Applying the dimensionless variables $c^* = (c - rc_0)/c_0$, $x^* = x/W$, $y^* = y/W$, and the Peclet numbers $Pe_x = UW/D_x$ as well as $Pe_y = UW/D_y$ into (3.21) leads to the dimensionless form:

$$\frac{\partial c^*}{\partial x^*} = \frac{1}{Pe_x} \frac{\partial^2 c^*}{\partial x^{*2}} + \frac{1}{Pe_y} \frac{\partial^2 c^*}{\partial y^{*2}} \quad (3.22)$$

With an arbitrary mixing ratio between the solute and the solvent $r : (1 - r)$ ($0 \leq r \leq 1$), the dimensionless boundary conditions of the inlets are:

$$f(y) = \begin{cases} c^* \Big|_{(x=0, 0 \leq y < r)} = 1 \\ c^* \Big|_{(x=0, r < y \leq W)} = 0 \end{cases} \quad (3.23)$$

For the convenience of solving the problems, the dimensionless concentration $c^* = (c - rc_0)/c_0$ is used. The boundary condition for inlets become:

$$f(y^*) = \begin{cases} c^* \Big|_{(x^*=0, 0 \leq y^* < r)} = 1 - r \\ c^* \Big|_{(x^*=0, y^*=r)} = 0 \\ c^* \Big|_{(x^*=0, r < y^* \leq 1)} = -r \end{cases} \quad (3.24)$$

The condition for full mixing at the outlet is:

$$c^* \Big|_{(x^* \rightarrow \infty, 0 \leq y^* \leq 1)} = 0 \quad (3.25)$$

The channel wall is impermeable. Thus, the flux at the wall should be zero:

$$\frac{\partial c^*}{\partial y^*} \Big|_{y^*=0,1} = 0 \quad (3.26)$$

The above models are depicted in Fig. 3.5. The dimensionless concentration distribution in the channel is:

$$c^*(x^*, y^*) = \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{\sin nr\pi}{n} \cos(n\pi y^*) \times \exp \left(\frac{\text{Pe}_x - \sqrt{\text{Pe}_x^2 + 4m \frac{\text{Pe}_x}{\text{Pe}_y}}}{2} x^* \right) \quad (3.27)$$

$n = 1, 2, 3 \dots$

where $m = n^2\pi^2/W^2$. With the Peclet number $\text{Pe} = UW/D$, the solution can be expressed as:

$$c^*(x^*, y^*) = \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{\sin nr\pi}{n} \cos(n\pi y^*) \times \exp \left(\frac{\text{Pe} - \sqrt{\text{Pe}^2 + 4m/G}}{2} x^* \right) \quad (3.28)$$

$n = 1, 2, 3 \dots$

where $G = 1 + h^2\text{Pe}^2/210$, $h = H/W$ is the aspect ratio of the channel. Figure 3.7 shows some typical results.

Since the zero-flux condition at the wall is the same as the symmetry condition and the trigonometric functions are convergent in the whole model, all the above solutions can be extended for micromixers with parallel multiple inlet streams.

Figure 3.7: The typical dimensionless concentration distribution with Taylor dispersion in the channel when $Pe=100$ and $h = 0.1$: (a) $r=2/3$ two inlets; (b) $r=1/2$ two inlets; (c) $r=2/3$ four inlets; (d) $r=1/2$ four inlets.

3.1.4 Discussions on mixing with the same viscosity

Ignoring the diffusion in flow direction, as reported by Holden et al. in [36], the above solutions have another form. For instance, equation (3.19) will have the following form:

$$c^*(x^*, y^*) = \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{\sin(nr\pi)}{n} \cos(n\pi y^*) \exp\left(-\frac{n^2\pi^2}{Pe} x^*\right) \quad (3.29)$$

$n = 1, 2, 3, \dots$

The difference between (3.19) and (3.29) lies only in the exponential terms. The exponent of (3.19) is $n^2\pi^2/[(1/2)(Pe + \sqrt{Pe^2 + 4n^2\pi^2})]$, while that of (3.29) is $n^2\pi^2/Pe$. When Peclet number Pe is very small or $Pe \ll n^2\pi^2$, the former expression is close to $n\pi$, which is different from that of (3.29). When Peclet number Pe is very large or $Pe \gg n^2\pi^2$, the former exponent approaches $n^2\pi^2/Pe$, which is the same as that of (3.29). Thus, the diffusion along flow direction can be ignored at high Peclet number Pe . However, it's important to note that the simplified model of Holden et al. is not accurate when Peclet number Pe is very small.

The mathematical expression of (3.29) is a sum of a product of three parts: the modulating coefficients, the cosine function and the exponential function. Corresponding to their effects in the solution, the exponential function determines the trend of the concentration distribution along flow direction and the cosine function determines the variation across the channel width. For rapid mixing, the absolute value of the exponent should be large. That means, a small Peclet number causes better mixing. Therefore, the Peclet number Pe is an important parameter of the mixing process in microchannels. In the case of only two inlets, according to the cosine function the highest concentration will appear near the wall. Thus, for evaluating the mixing length only the concentration at the wall needs to be considered. With $y^* = 0$, we get:

$$c^*(x^*, 0) = \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{\sin nr\pi}{n} \times \exp \left(-\frac{n^2\pi^2}{\frac{1}{2} \left(Pe + \sqrt{Pe^2 + 4n^2\pi^2} \right)} x^* \right) \quad (3.30)$$

$n = 1, 2, 3 \dots$

The concentration at the wall of the simplified solution (3.29) is:

$$c^*(x^*, 0) = \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{\sin nr\pi}{n} \exp \left(-\frac{n^2\pi^2}{Pe} x^* \right) \quad (3.31)$$

$n = 1, 2, 3 \dots$

Figure 3.8 shows the influence of the Peclet number on the concentration at the wall along the mixing channel. Based on the expression of the exponent in (3.29), Holden et al. introduced a dimensionless parameter $\kappa = x^*/Pe$ [36]. However, they did not elaborate further on this parameter. This parameter can be regarded as a dimensionless mixing length in microchannels. Assuming a channel length of L , the residence time in the channel is $t = L/U$. According to Einstein's theory [23], t is proportional to W^2/D . Thus, L is proportional to UW^2/D . Dividing x by UW^2/D results to the proportional factor $DL/UW^2 = x^*/Pe$. From system theory, the response function $\exp(-t/\tau)$ is considered as complete if $t \approx 3\tau$. Similarly, κ can be used for evaluating the extent of mixing. This dimensionless parameter is helpful for designing and evaluating T-mixers and its derivatives.

Figure 3.8: Normalized concentration along the dimensionless channel length at different Peclet numbers ($y^* = 0$).

3.2 Mixing with different viscosities

In the above models, the viscosities of solvent and solute are considered as equal and thus a uniform velocity can be assumed. However, the viscosity of the mixed streams are not equal in reality. With the different viscosities, a number of phenomena emerge in the mixing process. The most obvious phenomenon is that the velocity distribution in the channel is not uniform even in the case of a flat microchannel. Subsequently, the non-uniform velocity distribution contributes to a complex convection process and affects the concentration distribution. Due to the complexity of this problem, an approximate analytical model for velocity distribution is formulated and solved. The model ignores the diffusion between the two streams. The transport equation with the non-uniform velocity distribution is then solved numerically using finite control volume method.

3.2.1 Velocity distribution

As mentioned above, mixing with different viscosities is a complicated problem, which is strongly coupled between different fields. First, the velocity is not symmetrical along the

Figure 3.9: Geometry of the dimensionless model of the microchannel with two inlet streams.

flow direction, due to the spreading effect of the fluid. The different widths and velocities of the streams affect the mixing process significantly. Second, the mixing process changes the viscosities of the fluids, which in turn affect their spreading behavior and the velocity distribution in the channel. Thus, it is difficult to solve this problem analytically.

If diffusive mixing is negligible between the two species, the spreading behavior approaches the model of immiscible streams. Diffusion across the channel width can be neglected at the entrance of the channel or at a high Peclet numbers. From its definition, a high Peclet number can be achieved with a high stream velocity of a low diffusion coefficient. A very low diffusion coefficient means the fluids are immiscible. If the two streams in the microchannel (Fig. 3.1) are immiscible, the fully developed flow in the microchannel is governed by Navier-Stokes equations.

For better understanding of the model depicted in Fig. 3.1, the proved version is shown in Fig. 3.9. With the same dimensionless coordinate system used in the previous models and $z^* = z/W$, the Navier-Stokes equations for the regions 1 and 2 in Fig. 3.9 are:

$$\begin{aligned} \frac{\partial^2 u_1}{\partial y^{*2}} + \frac{\partial^2 u_1}{\partial z^{*2}} &= \frac{W}{u_0 v_1} \frac{\partial p^*}{\partial x^*} \\ \frac{\partial^2 u_2}{\partial y^{*2}} + \frac{\partial^2 u_2}{\partial z^{*2}} &= \frac{W}{u_0 v_2} \frac{\partial p^*}{\partial x^*} \end{aligned} \quad (3.32)$$

where v_1 and v_2 are the viscosities of the two streams, u_0 is a reference velocity, and $\partial p^*/\partial x^*$ is the pressure gradient along the x-axis. Similar to the methods used in 3.1.2,

with $P' = \frac{W}{u_0 v_1} \frac{\partial p^*}{\partial x^*}$ and $\beta = v_2/v_1$, the solutions of (3.32) have the forms:

$$\begin{aligned} u_1^* &= P' \left[\frac{z^{*2} - h^2/4}{2} + \sum_{n=1}^{\infty} \cos \theta z^* (A_{1n} \cosh \theta y^* + B_{1n} \sinh \theta y^*) \right] \\ u_2^* &= \frac{P'}{\beta} \left[\frac{z^{*2} - h^2/4}{2} + \sum_{n=1}^{\infty} \cos \theta z^* (A_{2n} \cosh \theta y^* + B_{2n} \sinh \theta y^*) \right] \\ n &= 1, 2, 3 \dots \end{aligned} \quad (3.33)$$

where $h = H/W$ and $\theta = (2n-1)\pi/h$. The non-slip boundary conditions are assumed for the walls:

$$u_1^*(0, z^*) = u_2^*(1, z^*) = 0 \quad (3.34)$$

At the interface of the two streams, the velocity and shear stress are assumed to be continuous:

$$\begin{aligned} u_1^*(r, z^*) &= u_2^*(r, z^*) \\ \left. \frac{\partial u_1^*}{\partial y^*} \right|_{y^*=r} &= \beta \left. \frac{\partial u_2^*}{\partial y^*} \right|_{y^*=r} \end{aligned} \quad (3.35)$$

where r is dimensionless interface of the two regions (see Fig. 3.9). Using the above boundary conditions and Fourier analysis, the coefficients in (3.33) are:

$$\begin{aligned} A_{1n} &= (-1)^{n+1} \frac{4h^2}{(2n-1)^3 \pi^3} \\ B_{1n} &= A_{1n} \frac{(1-\beta)(1-\cosh \theta) \sinh^2 r\theta + (1-\beta) \cosh r\theta \cosh \theta + \beta \sinh^2 r\theta}{(1-\beta) \sinh r\theta \cosh r\theta \cosh \theta - \sinh \theta (\cosh^2 r\theta - \beta \sinh^2 r\theta)} \\ &\quad - A_{1n} \frac{(1-\cosh \theta) \sinh r\theta \sinh \theta (\cosh^2 r\theta - \beta \sinh^2 r\theta) + \cosh^2 r\theta}{(1-\beta) \sinh r\theta \cosh r\theta \cosh \theta - \sinh \theta (\cosh^2 r\theta - \beta \sinh^2 r\theta)} \\ A_{2n} &= A_{1n} \frac{(1-\beta) \sinh r\theta \cosh r\theta \cosh \theta - \sinh \theta (\cosh^2 r\theta - \beta \sinh^2 r\theta)}{(1-\beta) \sinh r\theta \cosh r\theta \cosh \theta - \sinh \theta (\cosh^2 r\theta - \beta \sinh^2 r\theta)} \\ &\quad + A_{1n} \frac{-\beta \cosh \theta + (\beta-1) \cosh r\theta \cosh \theta - \beta \sinh^2 r\theta + \cosh^2 r\theta}{(1-\beta) \sinh r\theta \cosh r\theta \cosh \theta - \sinh \theta (\cosh^2 r\theta - \beta \sinh^2 r\theta)} \\ B_{2n} &= A_{1n} \frac{\beta \cosh \theta + (\beta-1) \cosh r\theta \cosh \theta + \beta \sinh^2 r\theta - \cosh^2 r\theta}{(1-\beta) \sinh r\theta \cosh r\theta \cosh \theta - \sinh \theta (\cosh^2 r\theta - \beta \sinh^2 r\theta)} \end{aligned} \quad (3.36)$$

With the same volumetric flow rate in each stream, the dimensionless width r of stream 1 (Fig. 3.9) can be estimated as:

$$r = \frac{1}{1+\beta} \quad (3.37)$$

Figure 3.10: The typical velocity profile in a microchannel with two immiscible streams with different viscosity ratios ($h = 0.14$): (a) $\beta = 1$, (b) $\beta = 2$, (c) $\beta = 3$.

Figure 3.10 shows the typical velocity distributions of two immiscible streams in a rectangular cross section of the microchannel. The viscosity ratios β are 1, 2, and 3, respectively. It can be seen clearly, that the equal viscosities lead to a velocity profile of a single phase. If the viscosities are different, the less viscous stream flows faster, while the more viscous stream is wider.

3.2.2 Numerical model for mixing

The numerical model is based on finite volume method (FVM) (Appendix A) and implemented in FORTRAN. This method divides the model into numerous control volumes (CVs). Up wind scheme was used for discretization of the governing equation. A two-dimensional model for equation (3.3) is formulated. Only the gray area in Fig. 3.9 is considered in the model. The different average velocities are given for the two regions and r was estimated using (3.37) with $\beta = 1.8$. The diffusion coefficients of each stream are

Figure 3.11: The typical concentration distribution in a flow channel of two miscible flows with different flow rates under the assumption, that the velocity field does not change due to the mixing process: (a) $1000\mu\text{l}/h$, (b) $750\mu\text{l}/h$, (c) $500\mu\text{l}/h$, (d) $250\mu\text{l}/h$.

1.0×10^{-9} and 1.8×10^{-9} , respectively. The channel width is $900\mu\text{m}$; the channel height is $50\mu\text{m}$ and the channel length is 50mm . In the simulation, 37,500 CVs were used and all the results converge after just 15 iterations. Figure 3.11 shows the typical results of the concentration distribution at different flow rates.

3.3 Hydraulic focusing and cross mixer

Hydrodynamic focusing is a simple but powerful method for handling a sample stream. The concept is based on the conventional cytometry. In conventional cytometry, the sample and the sheath flows are axis-symmetric. The focused sample in the center is passed through a detection region, where particles such as cells can be counted or separated. The miniaturized version of hydrodynamic focusing is planar. A basic device for hydrodynamic

focusing consists of a long channel with three inlets. The middle inlet is for the sample, while the sheath streams entry through the two other inlets. In other words, this configuration of microchannels can be considered as a special cross mixer.

Following, a rectangular microchannel with three inlets and one outlet is considered. The structure is similar to the one depicted Fig. 3.1. The flow direction is in x-axis. The cross section of the channel lies in the y-z-plane. The channel width is aligned with y-axis, while the channel height is parallel to the z-axis.

In this section, the analytical and numerical models are reduced to two dimensions. The model of hydrodynamic focusing of three immiscible flow is in the y-z-plane, while the model of diffusive dispersion is in the x-y-plane. In the model presented here, the sample flow and sheath are of different viscosities. The model is depicted in Fig. 3.12.

Figure 3.12: Analytical model of hydrodynamic focusing: (a) actual geometry, (b) the dimensionless model.

The model of hydrodynamic focusing consists of three streams: one sample stream sandwiched between two identical sheath streams, Fig. 3.12. Figure 3.12a shows the actual geometry of the channel cross section with the three streams. The width and height of the channel are $2W$ and H , respectively. The position of the interface is rW . Since the model is symmetrical regarding to the y-axis and the z-axis, only $1/4$ of the cross section needs to be considered. The velocity distribution u_1 and u_2 in the channel can be described by the

Navier-Stokes equations:

$$\begin{aligned}\frac{\partial^2 u_1}{\partial y^2} + \frac{\partial^2 u_1}{\partial z^2} &= \frac{1}{\eta_1} \frac{\partial p}{\partial x} \\ \frac{\partial^2 u_2}{\partial y^2} + \frac{\partial^2 u_2}{\partial z^2} &= \frac{1}{\eta_2} \frac{\partial p}{\partial x}\end{aligned}\quad (3.38)$$

where indices 1 and 2 describes the sample flow and the sheath flow, respectively. In Equation (3.38), η_1 and η_2 are the viscosities of the sample fluid and of the sheath fluid. Non-dimensionlizing the velocity by a reference velocity u_0 and the coordinates by W leads to the dimensionless model (Fig. 3.12a):

$$\begin{aligned}\frac{\partial^2 u_1^*}{\partial y^{*2}} + \frac{\partial^2 u_1^*}{\partial z^{*2}} &= \frac{W}{\eta_1 u_0} \frac{\partial p}{\partial x^*} \\ \frac{\partial^2 u_2^*}{\partial y^{*2}} + \frac{\partial^2 u_2^*}{\partial z^{*2}} &= \frac{W}{\eta_2 u_0} \frac{\partial p}{\partial x^*}\end{aligned}\quad (3.39)$$

With $P' = \frac{W}{\eta_1 u_0} \frac{\partial p}{\partial x^*}$ and $\beta = \eta_2/\eta_1$, and $\theta = (2n-1)\pi/h$, the solutions of Equation (3.39) have the forms at ($0 < y^* < 1$, $0 < z^* < h/2$):

$$\begin{aligned}u_1^*(y^*, z^*) &= P' \left[\frac{z^{*2} - h^2/4}{2} + \sum_{n=1}^{\infty} \cos \theta z^* (A_1 \cosh \theta y^* + B_1 \sinh \theta y^*) \right] \\ u_2^*(y^*, z^*) &= \frac{P'}{\beta} \left[\frac{z^{*2} - h^2/4}{2} + \sum_{n=1}^{\infty} \cos \theta z^* (A_2 \cosh \theta y^* + B_2 \sinh \theta y^*) \right] \\ n &= 1, 2, 3 \dots\end{aligned}\quad (3.40)$$

The non-slip conditions at the wall results in:

$$u_2^*(1, z^*) = 0 \quad (3.41)$$

The symmetry condition at z^* -axis is:

$$\frac{\partial u_1^*}{\partial y^*} \Big|_{y^*=0} = 0 \quad (3.42)$$

At the interface between the sample flow and the sheath flow, the velocity and the viscous rate are continuous:

$$\begin{aligned}u_2^*(r, z^*) &= u_1^*(r, z^*) \\ \frac{\partial u_1^*}{\partial y^*} \Big|_{y^*=r} &= \beta \frac{\partial u_2^*}{\partial y^*} \Big|_{y^*=r}\end{aligned}\quad (3.43)$$

For a flat channel ($h \ll 1$), the position of the interface can be estimated as:

$$r = \frac{1}{1 + 2\beta\gamma} \quad (3.44)$$

where $\gamma = Q_2/Q_1$ is the flow rate ratio between the one sheath stream and the sample stream.

A Fourier analysis with the above boundary conditions results in the coefficients of (3.40):

$$\begin{aligned} A_1 = & D [\beta \sinh \theta r \cosh^2 \theta r \cosh \theta - \sinh^3 \theta r \cosh \theta - \\ & (\beta - 1) \sinh^2 \theta r \sinh \theta \cosh \theta - (\beta - 1) \sinh^2 \theta r (\cosh \theta - \cosh \theta r) + \\ & (\beta - 1) \sinh \theta r \cosh \theta r \cosh \theta (\cosh \theta - \cosh \theta r)] / \\ & [\beta \cosh^2 \theta r \sinh \theta r \cosh^2 \theta - \sinh^3 \theta r \cosh^2 \theta - \\ & (\beta - 1) \sinh^2 \theta r \cosh \theta r \sinh \theta \cosh \theta] \end{aligned} \quad (3.45)$$

$$\begin{aligned} A_2 = & D [\beta \cosh^2 \theta r \cosh \theta - \sinh^2 \theta r \cosh \theta - (\beta - 1) \sinh \theta r \cosh \theta r \sinh \theta - \\ & (\beta - 1) \sinh \theta r (\cosh \theta - \cosh \theta r)] / \\ & [\beta \cosh^2 \theta r \sinh^2 \theta - \sinh^2 \theta r \cosh^2 \theta - \\ & (\beta - 1) \sinh \theta r \cosh \theta r \sinh \theta \cosh \theta] \end{aligned} \quad (3.46)$$

$$B_1 = 0 \quad (3.47)$$

$$\begin{aligned} B_2 = & D [(\beta - 1) \sinh \theta r (\cosh \theta - \cosh \theta r)] / \\ & [\beta \cosh^2 \theta r \cosh \theta - \sinh^2 \theta r \cosh \theta - \\ & (\beta - 1) \sinh \theta r \cosh \theta r \sinh \theta] \end{aligned} \quad (3.48)$$

where $D = (-1)^{n+1} \frac{4h^2}{(2n-1)^3 \pi^3}$. Figure 3.13 shows the typical velocity field inside the microchannel. The velocity of the sample flow is lower, if the sample flow is more viscous than the sheath flow ($\beta < 1$), Fig. 3.13a. If the viscosities are equal ($\beta = 1$), the flows behaves in the same way as a single phase, Fig. 3.13b. If the sheath flows are more viscous ($\beta > 1$), the sample flow is faster, Fig. 3.13c.

The diffusion effect in hydraulic focusing is also can be approximately modelled using this approach in the first section of this chapter for multiple streams. A more accurate

model can be obtained using numerical simulation similar to the methods mentioned in section 3.2.2.

Figure 3.13: The dimensionless velocity profile($h=0.14$, $\gamma = 1$): (a) $\beta = 0.5$, (b) $\beta = 1$, (c) $\beta = 2$.

3.4 Non-linear effects in micromixing

In microfluidic applications, due to the small channel size concentration gradients are much higher than those in macroscale. The dependence of diffusion coefficient on the concentration may have a bigger impact in microscale. A binary mixture of two species A and B is considered as ideal if the interactions between the pairs A-A, B-B and A-B are equal. However, in a real mixture the strength of the interactions differs from pair to pair depending on the concentration of each specie [134]. Thus, the diffusion coefficient of a specie depends on its own concentration.

A simple model of a binary mixture is considered between A and B, where A is the solute and B is the solvent. The diffusion coefficient of A at the maximum concentration $c = c_0$ is D_0 . At $c = 0$ the diffusion coefficient is aD_0 . The function of diffusion coefficient can be described as :

$$D(c) = D_0 \left[(1-a) \frac{c}{c_0} + a \right] \quad (3.49)$$

Considering the dimensionless concentration c^* used in the previous model, the diffusion coefficient is:

$$D(c^*) = D_0 [(1-a)(c^* + r) + a] \quad (3.50)$$

The coefficient a in Equation (3.49) and (3.50) describes the interaction between the molecules of the solute A and the solvent B:

- If $a = 1$, all interactions of A-A, B-B and A-B are equal, the mixing model is ideal and linear because of the constant diffusion coefficient $D = D_0$.
- If $a < 1$, the interaction A-B is stronger than A-A. Thus, it's more difficult for A to freely move in B than in A. The diffusion coefficient at low concentration of A is smaller.
- If $a > 1$, the interaction A-B is weaker than A-A. Thus, the diffusion coefficient at low concentration of A is larger.

The behavior described in Equation (3.49) was observed in many binary solutions [135, 136]. With this non-linear model, the Peclet number in [Equations (3.15), (3.19), and

(3.28)] has the form:

$$\text{Pe}(c^*) = \frac{\text{Pe}_0}{a + (1 - a)c^*} \quad (3.51)$$

where Pe_0 is the Peclet number evaluated with D_0 . Equation (3.10) is difficult to be explicitly solved with Equation (3.51). However, an iteration method can be used. The calculation starts with the linear solutions [Equation (3.15), (3.19), and (3.28)]. The next iteration determines the new Peclet numbers with Equation (3.51) using the concentration calculated in the previous iteration. The results often converge just after 3 iterations.

Figure 3.14 compares the results of the linear model ($a = 1$, Fig. 3.14a) with the non-linear models ($a < 1$, Fig. 3.14b) and ($a > 1$, Fig. 3.14c)

There are some other empirical estimations of the diffusion coefficient D . One is similar to the method based on arithmetic average [137], the other is geometric average [138].

3.5 Discussion and summary

3.5.1 The mixing in parallel micromixers

As discussed in 3.1.4 κ is most important parameter for T/Y-mixer. For convenience to discussing the problem, the expression of κ is rewritten here.

$$\kappa = x^*/\text{Pe} = DL/UW^2 \quad (3.52)$$

The parameter including the most two parameters that affect the mixing in the microchannel: dimensionless channel length and Peclet number Pe . The value of this number indicates how is the mixing at the outlet of the channel. General, T/Y-mixer can be regarded as the simplest parallel micromixer. Can this number be applied in parallel mixers? The answer is true but the channel width should be modified by dividing the number of the pairs of the inlets. The channel width should be the width of inlet pairs. For example, there are two parallel micromixers with the same channel width W : one is Y-mixer with one pair of inlets; the other is parallel micromixers with two pair of inlets. In the latter one, the channel width should be $W/2$ but W . The value of κ of latter one is only 4 times of that of former one. Therefore, in the latter one, the better can be obtained. From some meanings, the mixing is determined by local Pe or κ . It is especially useful to explain the mixing all other mixers (see the following subsections).

Figure 3.14: Distributions of concentration and concentration gradient across a microchannel ($Pe_0 = 50$): (a) ideal linear model $a = 1$, (b) non-linear model, stronger interaction between solvent and solute $a = 0.2$, (c) non-linear model, weaker interaction between solvent and solute $a = 5$.

Seeing from the other viewpoint, there are four most important parameters among the expression of κ : diffusion coefficient, channel length, channel width and velocity in three channel. As the practical mixer design, the species is often given. That is, the diffusion coefficient is often fixed. As to the velocity, for a good mixer, it should be arbitrary at some ranges. The rest for consideration is the geometry dimension: width and length of the mixers. To obtain good mixing, narrower channel width and longer channel length is desirable. From the expression, channel has a second order higher than that of channel length. Thus, it is more effective to narrow channel width than to extend channel length. In fact, in the parallel mixers, to add the inlet pairs decrease the channel width effectively.

3.5.2 The mixing in passive mixers

In the injection micromixers, they split one mixing inlet to many streams. The corresponding diffusion length (channel width) was decreased rapidly. Then the local Peclet number Pe was decreased rapidly, too. In the micromixers based on chaotic advection, the flow was split, broke-up, folded and stretched to get the small layer distribution pattern in the channel. The aim is same, to obtain small local Peclet number Pe . In the droplet micromixers, the mixing solutions were separated to many small droplets. The mixing was finished at small droplets. The essence of this method is same, to obtain small local Peclet number Pe by decreasing diffusion length (channel width) to droplets size. Therefore, small local Peclet number Pe is very critical to design passive micromixers.

3.5.3 Different viscosities mixing

The mixing with different viscosities is a very complex problem. It is a highly coupled problem between the different fields. During the mixing, it will change the viscosity of the flow. The flow distribution will change. Once the flow distribution is changed, it is natural that this change will affect the concentration distribution in the channel.

To discuss this problem, we can separate it to two problem: hydraulic spreading and mixing in the channel. From (3.44), the spreading can be affected by either changing the ratio of the flow rate ratios or viscosity ratio between the two mixing flows. It is different in same viscosity mixing. The boundary of the flow can be judged by flow rates. In mixing,

a unique character is the diffusion coefficient will change as the change of viscosity. All these make it difficult to analysis mixing with different viscosities. In the past works, a significant conclusion is clear: that smaller channel make rapid mixing is still effective in the mixing with different viscosities. This can be extended to local Peclet number Pe roughly but the problem is: it is very difficult to define local Peclet number Pe in this case.

3.5.4 Summary

The local Peclet number is the most important parameters for passive micromixers. To obtain small local Peclet number Pe is most important design aim and optimization parameter for passive micromixers design.

Chapter 4

Fabrication technologies and optical characterization techniques

This chapter describes the materials and methods for the prototypes and experimental results of the thesis. The chapter consists of two sections: the fabrication technologies and the optical characterization techniques. Polymethyl methacrylate (PMMA) was used for the fabrication of the micromixers described in this work. Laser micromachining was combined with lamination techniques using direct thermal bonding and adhesive bonding. The micromixers were then characterized by two optical characterization techniques: micro particle image velocimetry (microPIV) and scalar field measurement. These techniques allow the measurements of both velocity field and concentration field in a micromixer.

4.1 Fabrication technologies

The rapid development of life science and bio-technology attracts the attention of the micro electromechanical system (MEMS) community. Almost all micromachining techniques such as wet etching, dry etching, deep reactive ion etching, sputtering, and anodic bonding were used to fabricate microfluidic devices based on silicon and glass. However, due to the relatively large size of microfluidic devices and the high cost of traditional micromachining techniques, the cost of fabricating a microfluidic device in a small batch is relatively high. In addition, the devices for clinical and chemical applications are often disposable for a single use. This fact makes silicon-based technology even less attrac-

tive. Furthermore, silicon as a substrate is not always biocompatible. In contrast, polymers promise to be a low-cost substrate for microfluidic devices, which are also biocompatible.

In the past few years, many microfluidic devices were fabricated successfully using different polymers such as polydimethylsiloxane (PDMS), polystyrene (PS), polyethylene (PE), polyethyleneterephthalate (PET), polycarbonate (PC), SU-8 and polymethylmethacrylate (PMMA). Traditional plastic processing techniques such as casting, hot embossing, and injection molding were used widely. However, for research purpose these techniques were of high cost and require a long design cycle. These problems cause difficulties in research activities as well as delay in the development of new products.

Bonding quality is very important in microfluidics. The present bonding techniques for polymeric devices cannot always satisfy the stringent requirements of the applications. They are either not reliable or easily block the channel or of high cost.

In this work, laser micromachining was used as a rapid prototyping technique. Microfluidic devices were designed and fabricated in PMMA. An infrared carbon dioxide (CO_2) laser was used to engrave structures directly on the PMMA substrate. The geometry of these structures can be controlled precisely by adjusting the laser power and the scanning speed of the laser beam. The whole process was proved to be very quick. A micromixer can be completed in tens of minutes depending on the complexity of the structures.

There are two bonding methods for the micromachined polymeric parts. The first technique is adhesive bonding, which is based on the conventional lamination process. The second technique is direct thermal bonding, which was developed and optimized in this work. These bonding processes are simple and reliable. Direct thermal bonding under vacuum conditions was also investigated. The results indicate that a better bonding quality can be achieved under low-pressure conditions. The later experimental results show good device performances, which prove the success of these techniques.

4.1.1 PMMA as a substrate material

Early microfluidic devices were mostly fabricated in glass, quartz, or silicon. The fabrication techniques evolved from the standard photolithography and wet etching techniques of microelectronics. In MEMS-applications, silicon is regarded as a primary substrate be-

cause of its good mechanical properties. However, the electrical conductivity and optical opaqueness make silicon not always suitable to bio-applications. Glass and quartz are primary choice for devices in chemical analysis and bio-applications. These materials offer good chemical and optical properties. However, devices for chemical and biochemical analysis are expected to be disposable. Disposable devices need to be fabricated in large scale at a minimum cost. The cost of technologies based on glass or quartz is not low enough for the time being. Thus, polymers are low-cost alternatives with good chemical, electrical and optical properties. Polymers can be easily implemented for mass production. A number of fabrication techniques based on polymers were investigated in the past. Good review papers on polymer microfabrication are [139], [140] and [141].

Polymers are macromolecular materials, which are formed through polymerization reactions. In these reactions, the monomer units are cross linked in linear chains or in three-dimensional network chains to form a macromolecule. According to their properties, synthetic polymers can be categorized as thermoplastics, elastomers, and thermosets [142]. Polymethyl methacrylate (PMMA) is one of the thermoplastic polymers. Thermoplastic polymers are usually linear-linked and will soften when heated above the glass transition temperature. These polymers can be reheated and reshaped before hardening in its form for many times. Thermoplastic polymers can be crystalline or amorphous. Generally transparent polymers are non-crystalline, while translucent polymers are crystalline. PMMA has a non-crystalline structure. It has optical properties with a 92% light transmittance in the visible spectrum. PMMA also offers other excellent properties such as low frictional coefficient, high chemical resistance, and good electrical insulation. It can be cut, drilled, polished and cemented. Thus PMMA is a good substrate for microfluidic devices especially in biomedical applications.

4.1.2 Laser micromachining

Machining with laser transfers photon energy onto a workpiece. By melting and blowing away, or by direct vaporization, the laser removes material in the form of thermal energy or photochemical energy. The energy field is of great importance in laser machining. Four aspects of a laser influence the process of laser machining: time, space, frequency and mag-

nitude. Temporary modulation of energy field is an effective way to get better machining quality. The two basic questions for temporary modulation are:

- Is the laser continuous or pulsed?
- How high is the speed and acceleration of the laser beam?

These two factors control directly the quality of heat-affected zones on the workpiece. They also determine the speed of energy dissipation from the work piece, and further define the size of heat-affected zones.

Spatial properties of energy field is the second important factor in this process. They include the transverse working mode of the laser, the spot size, location and relative position between energy source and work piece.

Frequency is the next important parameter of the laser. Due to the good coherence, lasers have very narrow spectral width. Nowadays industrial lasers covers a wide spectrum from ultraviolet to infrared wavelengths. Micromachining with high frequency laser is determined mainly by photochemical reactions. Magnitude and frequency of a laser are closely related. The laser magnitude determines the intensity of the energy field on the workpiece.

Ultraviolet lasers are often used to realize microstructure in polymer materials. As mentioned above, laser ablation removes materials in the form of thermal energy or photochemical energy. This ablation mechanism is rather complex. During this process, some chemical bonds are directly damaged by photo absorption. Others bonds are damaged by thermal absorption of the molecules, or in simply melt. For more detailed discussions on the mechanism on laser ablation on PMMA, readers may refer to [143]. Although UV-laser is a good choice for laser ablation, the cost of UV-laser is the biggest problem for their use in research. In contrast to UV-laser, CO₂ laser is inexpensive. A CO₂ laser has a relative long characteristic wavelength of 10.6 μm . Thus, the ablation process depends more on thermal energy. The commercial Universal M- 300 Laser Platform (Universal Laser Systems Inc. <http://www.ulsinc.com>) was used for the prototypes presented in this thesis. The system uses a 25 Watt CO₂-laser cartridge, the maximum beam moving speed is about 640 mm/s.

The laser head is driven by a stepper motor. While moving across the workpiece surface, the laser forms a groove. As mentioned above, the ablation process depends mainly on thermal energy. Thus the cross section shape of the channel depends on the shape of the laser beam, its moving speed, power, and the thermal diffusivity of the workpiece. PMMA has a small thermal diffusivity. Thus, the shape of the channel is determined by laser.

In our experiment, the laser beam has a Gaussian-shaped energy distribution. This fact leads to a Gaussian-shaped cross section of the groove. The typical shape is shown in Fig. 4.1. The image is taken with scanning electronic microscope (SEM, Hitachi N3500S) at 20 Kilovolt and 80 times magnification. Klank et al. [144] found a linear relation between the channel depth and the laser power as well as the number of scanning passes. Besides these two parameters the influence of the head speed on the cross-section geometry is investigated. Channel widths and channel depths were measured using the above mentioned SEM-system. The results are shown in Fig. 4.2. For the results in Fig. 4.2a the beam speed is fixed at 4% of the maximum value, while the laser power is varied from 3% to 10.5% of the maximum power. The channel depths increase linearly with the laser power as observed by Klank et al. [144]. However, the relation between the channel width and the laser power is not linear. In Fig. 4.2b the laser power was kept at a constant value of 7%, while the beam speed is varied from 1.1% to 8%. We can observe that the channel widths and channel depths are inversely proportional to the beam speed.

4.1.3 Thermal direct bonding of PMMA

PMMA consists of chains of methacrylate monomers via the covalent bond between the molecules, which serve as the backbone in the polymer. The covalent bond is a strong connection in atomic level or atom-group level. A macromolecule is the basic unit of the polymers. Some bonds caused by unsaturated end groups also exist between the macromolecules. When PMMA is heated, the thermal degradation can be divided three steps:

- At about 105°C, the so-called glass transition temperature, PMMA starts to soften;
- At around 250°C unsaturated end groups starts to unzip;
- At above 300°C random chain scission appears, the structure is quickly damaged.

Figure 4.1: Typical cross-sectional view of a laser-machined microchannel in PMMA.

The weak head-group bonds between the macromolecules start to be damaged at about 150°C [145]. There is also another weak head-to-head bond, which quickly loses its stability at around 180°C [146]. Corresponding to this temperature behavior, PMMA does not soften gradually but step by step. All previous thermal bonding processes try to keep the temperature below the glass transition point (about 105°C) to protect pre-fabricated microstructures such as microchannels [144, 147]. However a higher temperature offers better bonding, because more bonds can be broken and reconnected after cooling. We found that although PMMA begins to soften in the range of 105°C to 180°C but the structural integrity still can be warranted by exerting a relative low bonding pressure. A temperature of 165°C and a pressure less than 20 kPa were used in our bonding process.

The experimental implementation of this bonding technique is simple. A controlled hot plate was used for the temperature regime. The PMMA-sheets are placed between the hotplate and an upper aluminium plate. Low bonding pressure can be adjusted by putting weights on top of the upper plate. For a better surface flatness of the PMMA-layers, two polished silicon wafers are placed between the PMMA-parts and the hot plate as well as the upper plate. Prior to the bonding process, the PMMA parts were carefully cleaned and rinsed in ethanol and DI-water. Fig. 4.3 shows the temperature regime of the bonding process. After bonding at 165°C , the bonded stack is annealed at 80°C for relieving stress.

Figure 4.2: Influence of laser power (a) and beam speed (b) on geometric parameters. The relative values are based on a maximum laser power of 25 W and a maximum beam speed of 640 mm/s. Circles and squares are measurement results, lines are fitting curves.

Figure 4.3: Temperature regime for thermal direct bonding of PMMA.

The bonding pressure is kept at around 20 kPa or less. The total process time is about 4 hours.

Figure 4.4 shows the cross-section of different bonded channels. The channels were fabricated by different laser powers as described in the previous section. After cutting and polishing, images of the cross sections were taken with an optical microscope. Almost no sign of the bonding interface can be observed. Despite of the relative high bonding temperature, the microchannels still keep their original Gaussian shapes. The results show that this bonding technique is very promising.

Figure 4.4: Cross sections of the bonded channels.

An ideal bonding process should be carried out in vacuum environment to avoid bubble trapping in the bonding surface. The bubble problem is significant for CO₂-laser-machined PMMA sheets because of the convex lines around a microchannel, Fig. 4.5. These lines can have heights ranging from 20 to 40 μm above the substrate surface. During the CO₂-laser ablation, the bulk material is vaporized and driven out of the machined channel. Some of the material remains loosely at the edge of channel and forms the convex line. These convex lines are usually melted and flattened during the thermal bonding process.

To get better bonding quality, the process was carried out in a commercial wafer bonder (EVG 501) for silicon wafers. The thermal regime is similar to that shown in Fig. 4.3, but the process was carried out in a vacuum environment. Considering the residual stress produced during the bonding process, an annealing step was implemented during the cooling phase. The annealing temperature was selected as 80°C. A clean and flat PMMA surface is required for a good bonding quality. Before bonding, the PMMA chips were cleaned using IPA and washed using ultrasonic washing machine further after rinsed in DI-water. To avoid bubbles trapped between the PMMA chips, a reasonable pressure is needed to be applied on the wafer stack. When 1 bar pressure was applied, the bubble hardly appeared. Another measure can improve the bonding quality is to design gas guides on the chips, which help to vent out the residual gas in the surface. Figure 4.6 compares the same area of a bonded device with and without bubbles.

Figure 4.5: Residual convex line around a laser-machined microchannel in PMMA.

To test the bonding quality, sample devices are characterized using micro particle image velocimetry (microPIV) technique which will be introduced in the following sections. The two test microchannels are depicted in Figure 4.7. The straight channel in Fig. 4.7a has a width of $140\ \mu\text{m}$ and a depth of $70\ \mu\text{m}$. The micro diffuser in Fig. 4.7b has widths changing from $210\ \mu\text{m}$ to $600\ \mu\text{m}$. Red polystyrene particles (Duke Scientific) with $1\text{-}\mu\text{m}$ diameter were used for tracking the flow. Figure 4.8 shows the results of the straight channel in Fig. 4.7a. Since the microscope is focused on the channel middle, the wide top section also appears in the image and acts as noise. However, a typical parabolic velocity profile can be observed. Results of the diffuser in Fig. 4.7b are shown in Fig. 4.8. The velocity change can be clearly observed. MicroPIV measurements validate the suitability of PMMA microchannels for fluorescent analysis and the good quality of our thermal direct bonding technique. This technique was also used to fabricate a micro analysis cartridge, Fig. 4.10.

4.1.4 Adhesive bonding

Adhesive bonding was used due to its short prototyping time. Using this technique, fully polymeric devices were fabricated and tested successfully in a short period. The

Figure 4.6: Bonding qualities: (a) with bubbles (low bond pressure, no vacuum), (b) without bubbles (high bond pressure, with vacuum)

fabrication is based on laser machining and adhesive lamination. There are three basic layers in a device: the top layer (PMMA) for optical access, the intermediate adhesives layer (Adhesives Research, Inc, ARclad 8102 transfer adhesive) for structure and bonding material, and the bottom layer (PMMA) for fluidic access and measurement positioning.

First, the three layers are cut and engraved using CO₂-laser as described above. Alignment holes are drilled by the same laser in all three layers. On the bottom layer, position marks are ablated for the convenience of later measurement. Through a customized alignment system, the three layers are sandwiched, Fig. 4.11. The adhesive layer thickness of 50 μm defines the channel height. Using this technique a mixing channel with 900 μm width and 50 μm length was fabricated for the experiments described in the next chapter. The three layers are positioned with the alignment holes and pressed for final bonding.

4.2 Optical characterization

Optical diagnosis is popular in microfluidics due to its advantage of high precision and non-invasive characteristics. Optical diagnosis combined with fluorescein techniques allow a number of measurements for microfluidic characterization. The following section discusses the two most important techniques: microPIV and scalar image measurement.

Figure 4.7: Fabricated PMMA-devices and the areas of interest for microPIV measurement: (a) straight channel, (b) diffuser.

4.2.1 Micro particle image velocimetry

Particle Image Velocity (PIV) is a powerful tool for visualization of complex velocity fields. Recently, PIV has been used in microscale for microfluidic diagnosis. MicroPIV can be considered as a further development of the conventional PIV. The major concept is to visualize the flow with the fluorescent tracer particles that faithfully move with the fluid. First, laser light illuminates and excites the fluorescent tracing particles. Subsequently the fluorescent tracing particles emit light in another wavelength. The particle images are recorded by film or digitally using charge couple device (CCD) camera. Finally, the flow field can be evaluated by correlation algorithms. The whole process of PIV is illustrated in Fig. 4.12. The technique of PIV can be divided in two basic steps: image acquisition and image processing.

Image acquisition system

Image acquisition is achieved with a set of microscale image acquisition system. The setup consists of three subsystems: an illumination system, an optical system, and an image acquisition system with its corresponding control software, Fig. 4.13.

Two different light sources are used in the system: a Mercury lamp and a set of pulsed

Figure 4.8: MicroPIV results in the straight channel.

Figure 4.9: MicroPIV results in the diffuser.

Figure 4.10: A complex PMMA analysis cartridge with an integrated micromixer and a micropump.

(a) Mixer prototype

(b) Channel cross section

Figure 4.11: The adhesive bonding mixer and channel cross section shape.

Figure 4.12: Flowchart of process of PIV.

Figure 4.13: Experimental setup for microscale image acquisition system.

Q-switched (quality switched) laser (Quantel, Quantel twins Q-switched double pulse Nd: YAG laser). The laser has a wavelength of 532 nm (green) and a maximum energy of 160 mJ per pulse. With a Q-switch inside the cavity, the laser can work in a triggered mode. The twin head system allows the realization of two laser pulses within a very short delay. The Mercury lamp was used for scalar field measurement such as characterization of mixing, because no synchronization is needed. For the measurement of velocity fields, the laser is required due to the need of precise synchronization. The technique of micro particle image velocimetry requires a short frame exposure time and a short delay between two frames. Thus, the camera shutter and the lasers should be precisely synchronized.

The system can work in different modes: single exposure in one frame, double exposure

in one frame, and double exposure in double frames. In the experiments described in this thesis, the mode of double exposures in double frames were used because of its high signal-to-noise ratios and the better quality of the cross-correlation technique. Switching of the two light sources is achieved by a mirror. When the mirror is placed parallel to the input light axis, the Mercury lamp is in use. When the mirror is placed at 45° to the the input light axis, the laser beam is in use.

The optical system consists of a Nikon inverted microscope (Model ECLIPSE TE2000-S) and a set of epi-fluorescence attachment. Filter cubes are the key components in the epi-fluorescence attachment. This attachment is very critical in the image acquisition for successful selection of the useful signal (emission light). There are three optical elements in a filter cube: excitation filter, dichroic mirror, and emission filter. Emission filters are used in both measurements to select more specifically the emission wavelength of the sample and to remove traces of excitation light. Dichroic mirror make both illumination and collection of fluorescent light possible. For the different requirements of the measurement, different objectives are needed. The selection of the objective is described in next section.

The image acquisition system consists of a camera and an image acquisition card in the control computer. This card was plugged in a personal computer (PC) as a peripheral component interface (PCI) card. The acquisition card with its corresponding software serves as the control unit of the whole system. Triggering the laser and camera allows strict synchronization of the whole measurement system. An interline transfer CCD camera (Sony ICX 084) was used for recording the images. The resolution of the camera is $640 \text{ pixels} \times 480 \text{ pixels}$, with 12 bits gray scale. The active area of the CCD sensor is $6.3 \text{ mm} \times 4.8 \text{ mm}$. The minimum inter-frame transfer time, and thus the fastest time delay for the two PIV images, is 300 ns. To ensure that the CCD camera works at its optimum temperature of -15°C , a cooling system is integrated in the CCD camera. In the mode of double exposure in double frames, the camera can record two frames of the flow fields and then digitizes them in to the same image buffer. The actual measurement setup is shown in Fig. 4.14

Image Processing

After image acquisition, if seeding satisfies the following requirements [148]:

Figure 4.14: The actual experimental setup for microscale image acquisition system.

- following exactly the motion of the fluid,
- not altering the flow or the fluid properties and
- not interacting with each other

are fulfilled, the fluid motion can be regarded as the same of tracing particles. The velocity can then be evaluated based on the two-dimensional correlation of two images at two different times.

There are two basic evaluation methods: auto-correlation and cross-correlation. Due to high signal noise ratio and no ambiguity of auto-correlation of a single image, cross-correlation is widely used as a standard algorithm for image evaluation.

Two images of the same fluid area are taken at two different times. They are first divided into many smaller regions called interrogation windows. The cross correlation of the same interrogation window of the two images reveals the averaged displacement of the particles:

$$\Phi(m,n) = \sum_{j=1}^q \sum_{i=1}^p f(i,j) \cdot g(i+m,j+n) \quad (4.1)$$

where $f(i, j)$ and $g(i, j)$ are the image intensity (gray-value) arrays of the two windows, j, j, m, n are indices for pixel positions. The velocity vector can be calculated from the evaluated displacement vector and the given time delay between the two images.

Tracing particles

From the above discussion, tracing is critical for microPIV experiments. Since PIV is an indirect measuring technique, the measurement highly depends on the particle velocity instead of fluid velocity. Thus, the dynamic properties of tracer particles have to be checked in order to avoid significant discrepancies between fluid and the particles. Furthermore, the final result highly depends on the quality of the images, which is highly dependent on the tracer particles optical properties. Based on these two points, this section emphasizes on dynamic and optical behaviors of the tracing particles.

Dynamic behaviors of tracing particles are first determined by the mismatch of the densities between the fluid and tracer particles. Assuming spherical particles in a viscous fluid at very low a Reynolds number, the estimated velocity lag U_s of a particle in the fluid is:

$$U_s = d_p^2 \frac{\rho_p - \rho}{18\nu} a \quad (4.2)$$

where d_p is the diameter of the particle, ρ_p is the density of the particle, ρ is the density of the fluid, ν is the dynamic viscosity of the fluid and a is the acceleration of the fluid. The response time τ_s of the particle is:

$$\tau_s = d_p^2 \frac{\rho_p}{18\nu} \quad (4.3)$$

Equations (4.2) and (4.3) suggest that the similarity between fluid density and particle density is necessary for the experiment. For example, when using microPIV for liquid flows with of 300-nm polystyrene latex spheres, the particle response time would be 10^{-9} second. This response time is acceptable for most cases.

The next important factor is the effect of Brownian motion. When the seed particles are small, the effect of Brownian motion is significant because the probability of unbalanced collisions between fluid molecules and particles becomes larger. This effect leads to two potential problems:

- Noise and error in velocity measurement and

- Uncertainty of the location of the tracing particles.

Generally, the spectrum of a particle due to Brownian motion consists of frequencies that are too high to be resolved fully. Thus, it is common that the velocity spectrum of a particle due to Brownian motion is modeled as Gaussian white noise. Thus particle's displacement can be averaged. If the time interval Δt is much larger than the particle's response time, the dynamics of Brownian motion displacement d_B is proportional to the product of diffusion coefficient D of particle and time intervals Δt :

$$d_B \propto D\Delta t \quad (4.4)$$

If a spherical particle subjects to Stokes drag law, the diffusion coefficient is given by Einstein as:

$$D = \frac{kT}{3\pi\eta d_p} \quad (4.5)$$

where k is the Boltzmann's constant, T is absolute temperature of the fluid, η is the dynamic viscosity of the fluid, and d_p is the particle diameter. If there is no Brownian motion, the displacements in x and y during the time of Δt should be:

$$\Delta x = u\Delta t \quad (4.6)$$

$$\Delta y = v\Delta t$$

where u and v are the time-averaged local fluid velocity in x and y components, respectively.

The relative errors ϵ_x and ϵ_y due to Brownian motion are then:

$$\begin{aligned} \epsilon_x &= \frac{\langle s^2 \rangle^{1/2}}{\Delta x} = \frac{1}{u} \sqrt{\frac{2D}{\Delta t}} \\ \epsilon_y &= \frac{\langle s^2 \rangle^{1/2}}{\Delta y} = \frac{1}{v} \sqrt{\frac{2D}{\Delta t}} \end{aligned} \quad (4.7)$$

where $\langle s^2 \rangle^{1/2}$ is the mean square distance of Brownian motion. These two equations show that the relative Brownian intensity errors decrease as the time of measurement increases. This fact gives a lower limit on the measurement time, because the measurement are dominated by uncorrelated Brownian motion. Olsen and Adrian [149] gave a detailed discussion on the Brownian motion and correlation in particle image velocimetry. They

introduced a complex dimensionless parameter to emulate the effect of Brownian motion on MicroPIV. This work suggested that optical parameters and particle's diameter would have the influence on the extent of the effect of Brownian motion on MicroPIV.

Since the fluid's and particle's motions are not strictly synchronous, the time lag leads to position error due to Brownian motion. This effect is significant when flows are slow and the particles are small under long exposure time. If Brownian displacement in image plane is small compared to the particle image diameter, or the diffusion time is much less than exposure time, the location uncertainty can be neglected.

The optical behavior of tracer particles consists of two main effects: scattering behavior and fluorescence. These two features concurrently exist and affect each other. In microPIV, fluorescent particles are more popular than non-fluorescent particles. There are two reasons for it. The first reason that is the fluorescent particle has a higher emission efficiency than the scattering light. The other reason that is the signal from fluorescent particles can be easily separated from excitation wavelengths, which clearly enhance the signal-to-noise ratio.

Fluorescence is a molecular phenomenon in which a substance absorbs light with a low wavelength and almost instantaneously emits light with a higher wavelength [150]. This process is known as excitation and emission. A special optical filter can be used to separate the excitation and emission wavelengths. The CCD camera can capture good images without having direct contact with the laser beam. The larger the difference between the two wavelengths, the better is the image quality. Therefore, a large band gap between the excitation and emission wavelengths is preferred for measurement.

After comparing different kinds of fluorescent tracing particles, Duke red of Duke Scientific Co., was selected for subsequent measurements. The maximum excitation wavelength and maximum emission wavelength of these particles are 542 nm (green) and 612 nm (red), respectively. The maximum excitation wavelength is very close to the laser wavelength of 532 nm and the maximum emission wavelength is far from the excitation wavelength. In our microPIV experiments the diameter of the particles is 3 μm .

Calibration of MicroPIV

Since the fully developed flow in microchannels has been well investigated both theoretically and experimentally, it is suitable for calibration of microPIV. An in-channel micro dispenser on printed circuit board (PCB) was used as microchannel. The channel widths of the two capillaries are $1700\mu\text{m}$ and $700\mu\text{m}$, respectively. The channel depth of $40\mu\text{m}$ is equal the thickness of the copper layer. The channel structure is covered by a glass slide, which allows optical access for the microPIV measurement. Figure 4.15 depicts the fabricated channel structures with the image windows.

Figure 4.15: Channel structure for calibration.

Figure 4.16: Calibration: (a) velocity vectors plot; (b) comparison the profile of flow velocity.

Water was driven by a syringe pump and $1\text{ }\mu\text{m}$ red fluorescent particle was used to seed the flow. With a $20\times$ objective, the images were captured by a CCD camera. The corresponding image size is $240\mu\text{m}\times 320\mu\text{m}$. Thus, the whole channel width of $700\mu\text{m}$ could not be observed. The two 24 mJ lasers were short in the interval of $450\mu\text{s}$ to excite the particles. The evaluation was carried out by PIVview (PivTec GmbH, Germany) and then EDPIV from Purdue University. A $32\text{ pixels}\times 32\text{ pixels}$ with an overlap ratio of 50% interrogation window, which satisfies the Nyquist sampling criterion, is used in the evaluation. The result can be seen in Fig. 4.16 and 4.17.

Using ANSYS/FLOTTRAN, a three-dimensional numerical model was set up to validate the measurement results. The velocity profile of a section was extracted and compared with the measurement results. In Fig. 4.16b, the line denotes numerical solution and circles denote the measurement results. In fluid measurement, to visualize the the complex flow and its change is one of most important driven force to develop PIV technique. Often, at the channel size changes, such in the transition area in window 1, Fig. 4.15, the accurate measurement of the flow field embody the advantage of micro-PIV technique for visualizing the complex flow field. Figure 4.18 comprises the stimulation results and measurement results Experimental and theoretical results agree well. The comparison indicates that our system works well can be used to characterize other microfluidic devices.

4.2.2 Scalar field measurement

Scalar field measurement based on the fluorescent technique. The same image acquisition setup was used. In the experiments, a fluorescent dye was utilized as solute and DI-water as the solvent. Fluorescent dye is a fluorescein disodium salt $\text{C}_{20}\text{H}_{10}\text{Na}_2\text{O}_5$, which has a commercial name of Acid Yellow 73 or C.I. 45350, diluted in DI-water and $10\times$ TBE buffer (for 1 Liters 108g Tris base, Tris (hydroxymethyl)-aminomethane 55g Boric acid 40mls 0.5M Ethylene Diamine Tetraacetic Acid (pH 8.0) autoclave for 20 min). The maximum absorption of the dye occurs at a wavelength of 490nm, while maximum emission occurs at the wavelength of 520nm. Two or three identical syringes (Hamilton gastight) were filled with the dye solution and the pure DI-water and placed on a same syringe pump (Cole-Parmer 74900-05, $0.2\text{ }\mu\text{L/h}$ - 500 ml/h , accuracy of 0.5%) The identical syringes

warrant the same flow rates of the inlet flows. Here 100 Watt Mercury lamp was used to illuminate the interested areas. In the measurements, a filter cube of type Nikon B-2A was used (excitation filter for 450-490 nm, dichroic mirror for 505 nm and an emission filter for 520 nm).

After the acquisition of the images, the concentration profiles were evaluated using a customized program written in MATLAB. First, the program removes the noise in the measured image with an adaptive noise-removal filter. For each pixel, a local mean value is calculated for a window of 5×5 pixels. The noise distribution is assumed to be the Gaussian distribution. Subsequently, a path with the known position across the channel is evaluated. The position across the channel was normalized against the dimensionless channel width $y^* = y/W$, while the measured pixel intensity I was normalized against the maximum $I_{\max}$ and minimum $I_{\min}$ of the intensity at the inlet :

$$I^* = \frac{I - I_{\min}}{I_{\max} - I_{\min}}. \quad (4.8)$$

The measured dimensionless intensity is assumed to be equal the dimensionless concentration c^* of the fluorescent dye ($I^* = c^*$). Figure 4.19 shows the typical intensity distribution in the mixing channel. Further results are discussed in the next chapter.

Figure 4.17: MicroPIV result in the transition area: (a) particle image, (b) evaluated flow field.

Figure 4.18: Transition area: (a) simulation results, (b) evaluated flow field.

Figure 4.19: Two typically intensity distribution (color coded) in the mixing channel at different positions.

Chapter 5

Experimental results and discussions

This chapter describes and discusses the results of the verification experiments for the models discussed in Chapter 3. Thus, this chapter is structured according to the sequence of Chapter 3. First, experimental results for mixing with the same viscosity are presented. Subsequently, results of mixing with different viscosities are reported. Finally, hydraulic focusing and nonlinear effects are characterized and discussed.

With the techniques described in Chapter 4, two types of micromixers were fabricated. The mixing channels have a width of $900\ \mu\text{m}$, a height of $50\ \mu\text{m}$ and a length of $55\ \text{mm}$, which satisfy the assumption of $H \ll W \ll L$ in Chapter 3. Also, to obtain a steady-state flow in the microchannel, a precision pump and precision syringes are selected (the detailed model can be seen the later experimental results). The standard Y-mixer was used to verify the model for mixing with 1:1 ratio and mixing with different viscosities. The second mixer has three inlets and a single outlet. This mixer was used to verify the model of mixing with an arbitrary ratio, mixing with multiple streams, and hydraulic focusing.

All the measurement reported in this chapter were carried out with a $4\times$ objectives. With a CCD sensor size of $6.3\ \text{mm} \times 4.8\ \text{mm}$, the size of an image pixel is $2.475\ \mu\text{m}$ and the size of the measured area is $1584\ \mu\text{m} \times 1188\ \mu\text{m}$. In microPIV measurements, fluorescent particles with a diameter of $3\ \mu\text{m}$ were used to trace the flow. The particles are diluted in DI-water. The solution was then filled in precision syringes, and pumped into the mixer using a syringe pump. The mixing measurement uses water with fluorescent dye and diluted glycerin as working liquids.

5.1 Mixing with the same viscosity

For mixing with the same viscosity, four groups of experiments were carried out:

- MicroPIV measurement for verification of an uniform velocity profile in the microchannel,
- Experiments with 1:1 mixing ratio,
- Experiments with 2:1 mixing ratio,
- Experiments with 2:1 mixing ratio and 3 inlets.

5.1.1 Measurement of the velocity field in the mixing channel

In Chapter 3, a uniform velocity or a Hele-Shaw flow was assumed for the mixing model. This model is acceptable, if the aspect ratio of the channel is small. In the experiments described here, the aspect ratio is 1/19. This relatively low aspect ratio satisfies the assumption of the theory. The velocity field was measured using microPIV to verify the above assumption.

The velocity fields of the entrance area and the fully developed flow at $x^* = x/W = 10$ were measured. The flow rate in the mixing channel was 200 $\mu\text{L/h}$. The laser beam had power of 30-mJ per pulse and a delay time of 3.5 ms. The interrogation window measures 32 pixels $\times$ 32 pixels. Figure 5.1 shows the results of the microPIV measurement. The results indicate that the entrance length at the junction is relatively short. The velocity profile in the mixing channel is uniform. Thus, the assumption of the analytical model is valid. The flow in the mixing channel can be considered as a two-dimensional Hele-Shaw flow.

5.1.2 Mixing with 1:1 ratio

Using the method described in Chapter 4, scalar intensity images of the fluorescent dye were obtained and then processed. For the convenience of the comparison with the theory model, the intensities are normalized according to:

$$I^* = \frac{I - I_{\min}}{I_{\max} - I_{\min}} - \frac{1}{2}. \quad (5.1)$$

Figure 5.1: Results of microPIV measurements: (a) at the entrance of the mixing channel, (b) in the mixing channel.

where I^* , I , $I_{\min}$, and $I_{\min}$ are the intensity values as explained in Section 4.2.2. Figure 5.2 shows the prototype of micromixer with two inlets. Figure 5.3 shows two typical three-dimensional color coded intensity images from the measurement.

Because of both unknown diffusion coefficient D_0 and the factor a , the non-linear theory presented in Section 3.4 was used for fitting the measurement results. While the solute side ($c^* = 1/2$) was used for finding D_0 , the solvent side ($c^* = -1/2$) was used for determining the factor a . First, the model uses $a = 1$ (linear model) for finding D_0 . The diffusion coefficient D_0 is found if the analytical solution matches the measurement at the solute side ($c^* = 1/2$). Subsequently, the diffusion coefficient D_0 determined in the first step was used in the model for finding a . The factor a is found if the analytical solution matches the measurement at the solvent side ($c^* = -1/2$). Using several measurements at different average velocities, the parameters $a = 0.4$ and $D_0 = 1.5 \times 10^{-9} \text{ m}^2/\text{s}$ could be found for the experiments presented in this section. Figure 5.4 compares the measurement results with the theoretical results using the above fitting parameters. It's to note, that the fluorescent dye used in this experiment was different from those in the following experiments because the TBE buffer was not added. The above estimated diffusion coefficient of this experiment is

Figure 5.2: Prototype of the micromixer with two inlets.

(a) $x^*=2.2$

(b) $x^*=47.8$

Figure 5.3: Three-dimensional intensity distribution in the captured image with $Q = 310 \mu\text{L/h}$, $u = 2090 \mu\text{m/s}$, $\text{Re} = 141.7 \times 10^{-3}$) at different positions.

Figure 5.4: Dimensionless concentration distribution in the mixing channel: (a) $Pe_0 = 153$ ($Q = 40 \mu\text{L/h}$, $u = 270 \mu\text{m/s}$, $Re = 18.3 \times 10^{-3}$); (b) $Pe_0 = 237$ ($Q = 62 \mu\text{L/h}$, $u = 418 \mu\text{m/s}$, $Re = 28.3 \times 10^{-3}$); (c) $Pe_0 = 1185$ ($Q = 310 \mu\text{L/h}$, $u = 2090 \mu\text{m/s}$, $Re = 141.7 \times 10^{-3}$).

Figure 5.5: Distribution of concentration and concentration gradient across the mixing channel at $x^* = 1, x^* = 12.8$ and $x^* = 48.1$ (solid lines are measured results, dashed lines are theoretical results with $D_0 = 1.5 \times 10^{-9} \text{ m}^2/\text{s}$ and $a = 0.4$): (a) $Pe_0 = 153$; (b) $Pe_0 = 237$; (c) $Pe_0 = 1185$.

not equal the values determined by all subsequent experiments.

Figure 5.5 compares the measured concentration profiles and concentration gradient profiles with the theoretical results with the fitting parameters mentioned above. It can be seen clearly that the non-linear model describes well the diffusive mixing process in the microchannel. The broadening band can be observed with the gradient profile. The band is thinner at a higher Peclet-number.

5.1.3 Mixing with an arbitrary ratio

In the experiments for mixing with an arbitrary ratio, a ratio of 2:1 was selected. The procedure and setup are the same as described in Section 5.1.2. The only difference is the use of a 10× TBE buffer in the fluorescent solution. The buffer increases the intensity of the fluorescent signal. In these experiments, a single three inlet mixer and the same mixing ratio 2 : 1 were used. Three identical syringes (Hamilton, 500 μL , GasTight) were used in the experiments. One syringe was filled with de-ionized (DI-)water, while the other two were filled with the diluted fluorescent dye. For mixing experiments with two streams, the syringe with DI-water connected to inlet 1, the other two syringes are connected to inlet 2 and 3. For mixing experiments with three-streams, the syringe with DI-water is connected to inlet 2, the other two syringes were connected to inlet 1 and inlet 3, Fig. 5.6.

Similar to 1:1 ratio mixing, an offset r was added to the normalized concentration profile. Therefore, the normalized formula for the intensity is:

$$I^* = \frac{I - I_{\min}}{I_{\max} - I_{\min}} - r. \quad (5.2)$$

In the experiment with 2:1 ratio, the value of the offset is $r = 2/(1 + 2) = 2/3$. Figure 5.7 shows the typical three-dimensional intensity distribution in the mixing channel.

Considering both unknown diffusion coefficient D_0 and the factor a , the non-linear mixing theory was used for fitting the measurement results. While the solute side was used for finding D_0 , the solvent side was used for determining the factor a . Using several measurement at different average velocities, the parameters $a = 0.5$ and $D_0 = 1.8 \times 10^{-9} \text{ m}^2/\text{s}$ could be found for the experiments. Due to the addition of 10× TBE buffer, these parameters are slightly different than the previous results for mixing with 1:1 ratio.

Figure 5.6: Prototype of three inlet micromixer.

Figure 5.7: Three-dimensional intensity distribution in the captured image with $U = 1.7$ mm/s and solute/solvent ratio of 2 : 1 at different positions: (a) two-streams mixing; (b) three-streams mixing.

Figure 5.8: Dimensionless concentration distribution for two-streams mixing (2:1 ratio) in the channels: a) $Pe_0 = 463$; b) $Pe_0 = 694$; c) $Pe_0 = 926$.

Figures 5.8 and 5.9 depict the results of mixing with two streams, while Figures 5.10 and 5.11 show the results mixing with three streams. In these figures, both concentration profiles and concentration gradients were evaluated. The experimental results are compared with their analytical counterparts. The results show clearly that the non-linear model describes well the diffusive mixing process in the microchannel. The broadening band can be observed with the gradient profile. The band is thinner at a higher Peclet number.

Figure 5.9: Distribution of concentration and concentration gradient across the channel at $x^* = 2.2$, $x^* = 13.3$ and $x^* = 47.8$ for three-streams mixing (ratio is 2:1) (solid lines are measured results, dashed lines are theoretical results with $D_0 = 1.8 \times 10^{-9} \text{ m}^2/\text{s}$ and $a = 0.5$): (a) $Pe_0 = 463$; (b) $Pe_0 = 694$; (c) $Pe_0 = 926$.

Figure 5.10: Dimensionless concentration distribution for asymmetric (ratio is 2:1) multiple mixing in the channels: (a) $Pe_0 = 347$; (b) $Pe_0 = 463$; (c) $Pe_0 = 579$.

Figure 5.11: Distribution of concentration and concentration gradient for asymmetric (ratio is 2:1) multiple mixing across the channel at $x^* = 4.4$, $x^* = 26.6$ and $x^* = 95.6$ (solid lines are measured results, dashed lines are theoretical results with $D_0 = 1.8 \times 10^{-9} \text{ m}^2/\text{s}$ and $a = 0.5$): (a) $Pe_0 = 347$; (b) $Pe_0 = 463$; (c) $Pe_0 = 579$.

5.2 Mixing with different viscosities

For mixing with different viscosities, DI-water acts as the fluid with low viscosity. Diluted glycerol with a volume ratio 1:4 (glycerol to DI-water at 25 °C) were used as the fluid with the high viscosity. The expected viscosity ratio is about $\beta=1.7$ -1.9. Viscosity measurement of the two liquids with a viscometer (Brookfield LVDV-I) results to a ratio of $\beta=1.7\pm0.1$. In the microPIV measurement, microspheres were added to DI-water and then to diluted glycerol with the same volume ratio, so that the same viscosity ratio can be expected. In the measurement of diffusive mixing, the dye/water solution was used as the solute with low viscosity and the diluted glycerol was used as the solvent with high viscosity.

5.2.1 Measurement of the velocity field in the mixing channel

The measurement was carried out with a $4\times$ objective and $3\text{-}\mu\text{m}$ particles. The delay time were adjusted to satisfy the requirements of PIV algorithms. Figure 5.12 shows the typical results of the microPIV measurement. In order to investigate the change of the velocity profile along the mixing channel, 26 positions of interest along the channel were measured. Figure 5.13 shows the measured velocity profiles along the microchannel.

Figure 5.14 compares the normalized velocity profile with the analytical model derived from Chapter 3. The velocity is normalized against the average velocity u_0 across the channel width. The analytical results was calculated with the viscosity ratio $\beta=1.8$.

At the channel entrance the velocity shows a similar behavior compared to the analytical model (Fig. 5.14). The higher measured velocities of the less viscous stream may be caused by the entrance effect. The measured division position is about $r=0.38$. However, the division between the two streams disappear as they tend to have the same velocity. The reason for this effect is the result of the mixing process at the end of the channel. The two streams are converted into a single phase if they are totally mixed. The velocity gradients in Fig. 5.12b show clearly this behavior.

Figure 5.15a shows the velocity profile at $x^*=11.1$ at different flow rates in the two streams. It can be clearly seen that the maximum velocities of both sides increase proportionally with the flow rates.

Figure 5.12: Typical results of a micro-PIV measurement (the flow rates of glycerol and water are both $750 \mu\text{L/h}$).

Figure 5.13: Velocity profile along the channel length (the flow rate of each stream is $750 \mu\text{L/h}$).

Figure 5.14: MicroPIV results at the entrance $x^*=2.2$ and at the end of the channel $x^*=55.5$ (the flow rate of each stream is $750 \mu\text{L/h}$, analytical results are calculated with $\beta=1.8$): (a) normalized velocity profile, (b) normalized velocity gradient.

Figure 5.15: Velocity profile along the channel length (the flow rate of each stream is 750 $\mu\text{L/h}$).

Figure 5.16: Typical results of the florescent measurement (the flow rates of glycerol and water are both $250 \mu\text{L/h}$): (a) at the entrance $x^*=2.2$, (b) at the channel end $x^*=55.5$.

5.2.2 Measurement of the concentration field

Mixing measurements used DI-water diluted with fluorescent dye and diluted glycerin as working fluids. Figure 5.16 shows the typical three dimensional images of the florescent measurements. Equation (5.2) also was used. For dimensionless evaluation, the dimensionless interface position $r=0.38$ was taken from the from microPIV measurement.

The numerical model for this problem was formulated with 37500 control volumes. The model consists of two streams with different diffusion coefficients and velocities. The velocities are taken from the microPIV measurement described above. They are $3831 \mu\text{m/sec}$ for dye/water solution and $2146 \mu\text{m/sec}$ for diluted glycerol. The diffusion coefficient $D_0=1.8 \times 10^{-9} \text{ m}^2/\text{s}$ in dye/water solution was determined in the previous section. Since the diffusion coefficient is inversely proportional to the viscosity, the diffusion coefficient of diluted glycerol is assumed to be $1.0 \times 10^{-9} \text{ m}^2/\text{s}$. The non-linear effect caused by the different diffusion coefficients in each stream is considered in the analytical model by the concentration dependent coefficient from Equation (3.50). A constant average velocity and consequently a constant Peclet number $\text{Pe}=1678.25$ was assumed for a flow rate of $250 \mu\text{L/h}$ in each stream.

Figure 5.17 compares the dimensionless concentration distribution in the micro channel obtained from the measurement, the numerical model and the analytical model. Figure 5.18 shows the concentration distributions of the fluorescent dye at the entrance ($x^*=2.2$) and

at the end of the channel ($x^*=55.5$). The influence of the different stream velocities can be clearly observed here. While the numerical model with the different velocities agrees relatively well with the measurement, the analytical model cannot represent the mixing effect in the microchannel. Because of the smaller average velocity assumed for the model, diffusion across the dye/water stream is much higher than in reality.

Figure 5.19 compares the dimensionless concentration distribution in the microchannel obtained from the measurement, the numerical model and the analytical model at the flow rate of $500 \mu\text{L/h}$. In the numerical model, the velocities are $7662 \mu\text{m/sec}$ and $4932 \mu\text{m/sec}$ for dye/water solution and diluted glycerol, respectively.

In the analytical model, a constant Peclet number $Pe=3356.5$ was assumed for each stream. Figure 5.20 compares the dimensionless concentration distribution in the microchannel obtained from the measurement, the numerical model and the analytical model at the flow rate of $750 \mu\text{L/h}$. In the numerical model, the velocities are $11463 \mu\text{m/sec}$ for dye/water solution and $6438 \mu\text{m/sec}$ for diluted glycerol. The diffusion coefficients are the same as the above work. A constant Peclet number $Pe=5034.5$ was assumed for each stream in the analytical model.

Figure 5.21 compares the dimensionless concentration distribution in the micro channel obtained from the measurement, the numerical model and the analytical model at the flow rate of $1000 \mu\text{L/h}$. In the numerical model, the velocities are $15324 \mu\text{m/sec}$ and $8584 \mu\text{m/sec}$ for dye/water solution and diluted glycerol, respectively. A constant Peclet number $Pe=6613$ was assumed for each stream in the analytical model.

5.3 Hydraulic focusing

In characterization experiments of hydraulic focusing, both microPIV measurement and fluorescent measurement were carried out with a $4\times$ objective. Three identical syringes with a total volume $1000 \mu\text{L}$ each were used. The same working fluids (DI-water and diluted glycerin) described in the previous section were used. Two groups of experiments are carried out:

- Water in the sample flow and diluted glycerin in the sheath flows;

Figure 5.17: Dimensionless concentration of the fluorescent dye in the microchannel (the flow rates of glycerol and water are both $250 \mu\text{L/h}$): (a) measurement, (b) numerical model, (c) analytical model.

Figure 5.18: Normalized concentration distribution at the entrance $x^*=2.2$ and at end of the channel end $x^*=55.5$ (the flow rates of glycerol and water are both $250 \mu\text{L/h}$)

- Diluted glycerin in the sample flow and water in the sheath flows.

5.3.1 Measurement of the velocity field in the mixing channel

Figure 5.22 shows the measured velocity profiles at the same position ($x^* = 22.2$), but different flow rates. The y-axis was normalized with the channel width $2W$. The flow rates of the sample flow (water) and the sheath flows (diluted glycerol) were kept equal ($\kappa = 1$) using the same syringe pump for three identical syringes. It's clear from Fig. 5.22a that the higher flow rates cause a higher velocity in each stream. Normalizing by the average velocity across the channel u_0 , the velocity profiles and the interface position agree well with the theory ($\beta = 1.8$, $\kappa = 1$, $r = 0.22$ after (3.20)), Fig. 5.22b. However, the smaller gradients at the interface (Fig. 5.22c) were caused by diffusive mixing of glycerol and water. Furthermore, the evaluation grid of $32 \text{ pixels} \times 32 \text{ pixels}$ may decrease further the actual velocity gradients.

Figure 5.23a compares the normalized velocity profile at the entrance ($x^* = 4.4$), at the

(a) Measurement

(b) Numerical model

(c) Analytical model

Figure 5.19: Dimensionless concentration of the fluorescent dye in the microchannel (the flow rates of glycerol and water are both $500 \mu\text{L/h}$): (a) measurement, (b) numerical model, (c) analytical model.

(a) Measurement

(b) Numerical model

(c) Analytical model

Figure 5.20: Dimensionless concentration of the fluorescent dye in the microchannel (the flow rates of glycerol and water are both $750 \mu\text{L/h}$): (a) measurement, (b) numerical model, (c) analytical model.

(a) Measurement

(b) Numerical model

(c) Analytical model

Figure 5.21: Dimensionless concentration of the fluorescent dye in the microchannel (the flow rates of glycerol and water are both $1000 \mu\text{L/h}$): (a) measurement, (b) numerical model, (c) analytical model.

Figure 5.22: MicroPIV results with water in the sample stream at different stream flow rates ($\beta = 1$, $\gamma = 1.8$, $x^* = 22.2$): (a) velocity profiles, (b) normalized velocity profiles, (c) normalized velocity gradients.

Figure 5.23: MicroPIV results water in the sample stream at the entrance ($x^* = 4.4$) and at the end of the microchannel ($x^* = 111.1$) ($\beta = 1.8$, $\gamma = 1$, $750 \mu\text{l/h}$): (a) normalized velocity profiles, (b) normalized velocity gradients.

channel end ($x^* = 111.1$) with the analytical result. At the channel entrance the velocity shows a similar behavior compared to the analytical model. However, the division between the two streams disappear as they tend to have the same velocity. The reason for this effect is the mixed area at the end of the channel. The two streams are converted into a single phase if they are totally mixed. The velocity gradients become flatter at the channel end, Fig. 5.23b.

The same measurements were carried out for the second case. Diluted glycerol works as the sample flow, while the sheath streams are water flows. Figure 5.24 shows the results at the same position ($x^* = 22.2$) with different stream flow rates. As predicted with the analytical model, the small viscosity ratio $\beta = 1/1.8 < 1$ leads to a slower sample flow. The sample flow also occupies more place of the channel width ($r = 0.47$, according to Fig. (5.22)). The influence of diffusive dispersion on the velocity field can also be observed in this case, Fig. 5.24b.

The results shown in Figures 5.22, 5.23, 5.24 and 5.25 indicate clearly the influence

Figure 5.24: MicroPIV results with diluted glycerol in the sample stream at different stream flow rates ($\beta = 1/1.8$, $\gamma = 1$, $x^* = 22.2$): (a) velocity profiles, (b) normalized velocity profiles, (c) normalized velocity gradients.

Figure 5.25: MicroPIV results with diluted glycerol in the sample stream at the entrance ($x^* = 4.4$) and at the end of the microchannel ($x^* = 111.1$) ($\beta = 1/1.8$, $\gamma = 1$, $750 \mu\text{l/h}$): (a) normalized velocity profiles, (b) normalized velocity gradients.

of diffusive dispersion on the velocity field. Mixing between diluted glycerol and water leads to a change in viscosities, which in turn affects the velocity field. At high Peclet numbers (high velocity or low diffusion coefficients) and at the channel's entrance, the measured results approach the analytical model of hydrodynamic focusing of immiscible liquids. Diffusive dispersion of the two cases above will be investigated by measurement with fluorescent dyes described in the next section.

5.3.2 Measurement of the concentration field

Figure 5.26 shows the normalized concentration of the first case with water in the sample stream at the entrance ($x^* = 4.4$, Fig. 5.26a) and at the end of channel ($x^* = 106.7$, Fig. 5.26b). Here, the measurement results are compared both the analytical and numerical results.

The diffusion coefficient in dye/water solution was determined in the above works and

Figure 5.26: Normalized concentration of fluorescent dye with water in the sample stream (a) at the entrance ($x^* = 4.4$) and (b) at the end of the microchannel ($x^* = 106.7$).

is $D_{0,water} = 1.8 \times 10^{-9} \text{ m}^2/\text{s}$ and the diffusion coefficient of diluted glycerol was assumed to be $D_{0,glycerol} = 1.0 \times 10^{-9} \text{ m}^2/\text{s}$. The non-linear effect caused by the different diffusion coefficients in each stream was also considered. A constant average velocity of $4629.6 \mu\text{m}/\text{s}$ and consequently a constant Peclet number of $Pe = 2314.8$ was assumed for all streams. The interface position was taken from (3.46).

Similar to the case of mixing with different viscosities and two streams, the numerical model for the problem was formulated with 37500 control volumes. The model consists of three streams with different diffusion coefficients and velocities. The velocities are taken from the microPIV measurement as described above. They are $5823 \mu\text{m}/\text{sec}$ for dye/water solution and $2912 \mu\text{m}/\text{sec}$ for diluted glycerol in the first case, Fig. 5.26. In the second case with water in the sheath flows, the velocity of glycerol and water are $3967 \mu\text{m}/\text{sec}$ and $6953 \mu\text{m}/\text{sec}$, respectively.

The influence of the different stream velocities can be observed clearly in Figures 5.26 and 5.27. While the numerical model with the different velocities agrees relatively well with the measurement, the analytical model cannot represent the mixing effect in the microchannel. Because of the smaller average velocity assumed for the model, diffusion across the dye/water stream is much higher than in reality.

Figure 5.27: normalized concentration of fluorescent dye with diluted glycerol in the sample stream (a) at the entrance ($x^* = 4.4$) and (b) at the end of the microchannel ($x^* = 106.7$).

5.4 Uncertainty analysis

5.4.1 Velocity measurement

In the velocity measurement, the uncertainty comes from: (1) the uncertainty of driven pump; (2) the uncertainty from Brownian motion of the particles; (3) the uncertainty from PIV algorithm.

The pump used in the experiments has accuracy of 0.5% (<http://www.coleparmer.com/>). That is the certainty is 99.5%. According to (4.2), the velocity lag does not exist in the experiments in thesis because the flow is steady state, there is no acceleration in the flow. For errors introduced by Brownian motion, when the velocity is about 1 ms, the velocity is about few mm/s (such as 6 m/s in Fig. 5.12) and the particle size is 3 micron the errors is less than 0.3% according to (4.7). That is the certainty is larger than 99.7%. As to the uncertainty from PIV algorithm, it is less than 5% when the experiments satisfied the requirements specified in [151]. Therefore, the total uncertainty is $1 - 99.5\% \times 99.7\% \times (1 - 5\%) = 5.8\%$.

5.4.2 Concentration measurement

The uncertainty of concentration measurement main comes from the noise background. However, during the measurement, only the first line has a strict known value. Thus, the uncertainty analysis focuses on the first line. A standard derivation was calculated at the first lines. The results indicates the uncertainty is less than 5%.

Chapter 6

Novel mixer designs

According to the discussion in Chapter 2, passive mixers are preferred in analytical chemistry and bio assays. New mixer designs are needed to suit the low-Reynolds-number working condition. This chapter proposed two novel designs of micromixers. The first design is based on two-phase hydraulic focusing. The second design is based on twisting the mixed flows in the channel. Furthermore, a novel method was developed to realize the three-dimensional channel with a simple and rapid fabrication process. Both of these designs are passive and can work at low Reynolds numbers. Characterizations were carried out to evaluate the performance of the two designs.

6.1 Two-phase hydraulic focusing for rapid mixing

6.1.1 Design principle and theories

In Section 3.1.4, a dimensionless mixing length κ for parallel mixing was defined as:

$$\kappa = x^*/\text{Pe} = \frac{LD}{UW^2} \quad (6.1)$$

where Pe is the Peclet number, L is the length of the mixing channel, D is the diffusion coefficient, U is the averaged velocity of the flow, and W is the width of the mixing channel. In (6.1), κ is inversely proportional to two orders of W . The simplest method is to decrease the channel width as proposed in [52]. However, due to the limitation of the fabrication technique, decreasing the channel width means increasing the fabrication cost. An alternative

method is to adopt hydraulic focusing [62]. But in this case the sample would diffuse into the sheath flows. Furthermore, the focusing ratio only can be adjusted by changing the flow rate, which limits the focusing ratio of sheath flow and sample flow significantly.

To overcome these drawbacks of conventional hydraulic focusing, a design of micromixer based on two-phase hydraulic focusing was proposed in this thesis. In this design, the mixing flows are immiscible to sheath flows. Thus, the sample will not diffuse into the sheath flow. The mixing flow width is adjusted through adjusting the focusing ratio. The focusing ratio can be adjusted by either changing the ratio of the flow rate ratios or viscosity ratio between sheath flows and mixing flows.

The microchannel under consideration is similar to that of hydraulic focusing in Section 3.3. The channel has four inlet streams (see Fig. 6.1). The middle two are sample streams consisting of a solvent (white arrow in Fig. 6.1) and a solute (black arrow in Fig. 6.1). The rest are sheath streams (arrows with shadowed lines in Fig. 6.1) for focusing the mixing streams. The sheath flow and the mixing flow are immiscible. For the same reason in Section 3.3, the two-phase focusing is modelled in a cross section plane. The flow velocity distribution is studied in the y - z -plane with the flow direction in x -axis.

Figure 6.1: Physical model with its coordinate

The model of two-phase focusing consists of four streams: the two mixing streams sandwiched between two identical sheath streams. But from the point of view of the phase difference, the solvent and solute can be treated as a single phase. Thus, the same figure (Fig. 3.12) in Section 3.3 can be applied to describe this problem. Because the sheath flow and mixing flow are immiscible. They satisfy strictly the assumptions in Section 3.3. Therefore, Equation (3.39) can be used to describe two-phase focusing. The solution is Equation (3.40).

Figure 6.2: Models for convective-diffusive mixing in the channels: (a) the physical model, (b) the dimensionless model.

Convective-diffusive mixing

By introducing $Pe = 2rWU/D$, a two-dimensional model as depicted in Figure 6.2 can be established. Using the same method in Section 3.1.3 and the dimensionless concentration distribution is:

$$c^*(x^{**}, y^{**}) = \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{\sin n\pi/2}{n} \cos[n\pi y^{**} + \pi/2] \times \exp \left[\left(Pe - \sqrt{Pe^2 + 4n^2\pi^2 x^{**}} \right) / 2 \right] + \frac{1}{2} \quad (6.2)$$

$$n = 1, 2, 3 \dots$$

6.1.2 Experiments and results

A fully polymeric prototype was fabricated using the method described in Chapter 4, Fig 6.3. The channel dimension are the same as those of the prototype in Fig. 5.2. For characterization, the same method (Section 4.2.2) and instruments (Fig. 4.13) were used. To distinguish the interface of the two-phase flow, besides Mercury lamp for excitation of the florescent dye, an extra lamp with phase contrast condenser was used to illuminate the whole area of interest. Figure 6.4 gives some typical images taken along the channel. The prepared liquids (dye and DI-water) were primed into the microchannel using two precision syringes (Hamilton GasTight 500 μ l). The sheath flows (silicone oil with different

Figure 6.3: A micromixer with hydraulic focusing.

viscosities) also were primed using these precision syringes (Hamilton GasTight 1000 and 250 μl , respectively). The fluorescent measurement were carried out with a $20\times$ objective. The CCD sensor has a size of $6.3\text{ mm} \times 4.8\text{ mm}$ and a resolution of $640\text{ pixels} \times 480\text{ pixels}$. Thus, the size of an image pixel is $0.495\text{ }\mu\text{m}$, while the total size of the measured area of interest is $316.8\text{ }\mu\text{m} \times 237.6\text{ }\mu\text{m}$.

Figure 6.4: Typical intensity distribution along the channel: (a) two-dimensional gray scale image, the darker areas are the sheath flows and the lighter area in the middle is the mixing flow; (b) three-dimensional color mapped image.

To evaluate the extent of the mixing process, the concept of mixing index (M.I.) or

Figure 6.5: Typical normalized concentration distribution along the channel (Pe=1111, Re=0.76): (a) measurement results; (b) theoretical results.

degree of mixing was introduced:

$$\text{M.I.} = 1 - \frac{\sqrt{\frac{\sum_{i=1}^n (I_i^* - \mu)^2}{n}}}{\mu} = 1 - 2\sqrt{\frac{\sum_{i=1}^n (I_i^* - 0.5)^2}{n}} \quad (6.3)$$

where I_i^* is local normalized intensity; i is the index; μ is average value of local normalized intensity, in our experiments $\mu=0.5$. The diffusion coefficient in dye/water solution was determined in our previous works and is $D_0 = 1.5 \times 10^{-9} \text{ m}^2/\text{s}$ (Chapter 5). The non-linear effect caused by the different diffusion coefficients in each stream is considered in the analytical model by the concentration dependent coefficient. A constant average velocity was assumed for all streams. The width of mixing was taken from Equation (3.44) and adapted according to experimental results and for theoretical model. Figure 6.5 shows one typical normalized measurement concentration distribution with its corresponding theoretical results along the channel.

Two groups of experiments were carried out. In one group, the same mixing flow rate of $150 \mu\text{l/h}$, the focusing flow rate ratio and viscosities were adopted to change the mixing width, or the value of r in Equation (3.39). Silicone oils with different viscosities 100 cSt and 10 cSt were used. For both silicone oils, different focusing flow/sheath rates of $300 \mu\text{l/h}$ and $75 \mu\text{l/h}$ were used. In the second experiment group, only silicon oil viscosity of 10 cSt was used. The different mixing flow rates were used with the same flow rate ratio between sheath flows and mixing flow of 2. Figure 6.6a shows the mixing index profile along the channel with different focusing flow rate ratios at the same mixing flow rate. And Fig.

Figure 6.6: The comparison of mixing index profile between experimental results and theoretical results at different focusing flow rate ratios and viscosities with the same mixing flow rate ($150 \mu\text{l/h}$, $Pe=1111$): (a) experimental results; (b) the corresponding theoretical results.

Figure 6.7: The comparison of mixing index profile between experimental results and theoretical results at different mixing flow rates at the same focusing flow rate ratio and viscosity: (a) experimental results; (b) the corresponding theoretical results.

6.7a shows the mixing index profile along the channel at different mixing flow rates with the same focusing flow rate ratio. Figure 6.6b and 6.7b show the corresponding theoretical results. Due to many factors in the measurements, the mixing index could not achieve to the ideal value of 1. We found that even at the entrance for the dye inlet the uniform of dye only be 0.95. Therefore, a correction coefficient of $1/0.95$ was used in the depicted results. At high focusing flow rates and high viscosity with same mixing flow rates, mixing efficiency is significantly higher. In the other group of experiments, at different mixing flow rates with the same focusing flow rate and viscosity, mixing efficiency is dominated by the Peclet-number as predicted with the theory.

6.2 Three-dimensional twist switching micromixer

This section presents a three-dimensional micromixer with twist switching flow. The three-dimensional structure was formed by laser micromachining and direct bonding of two PMMA sheets. The experimental results show that switching of the flow appeared at certain Reynolds numbers and enhanced the mixing significantly. In microPIV experiments, there are evidences indicating the existence of flow circulating in the channel. Furthermore, a Y-mixer with the same channel width, depth and length was fabricated and characterized to compare the mixing efficiency under the same conditions. The comparison proves the advantage of the new micromixer.

6.2.1 Design and fabrication

From the previous analysis in section 3.5, better mixing can be achieved by:

- Reducing the channel width to decrease the mixing path,
- Increasing contact surface between solvent and solute,
- Creating chaotic advection to improve transversal species flow, which is not possible in a conventional Y-mixer.

However, the method to reduce channel width is limited in many cases due to technical reasons such as limitation of fabrication technique. The Peclet number cannot be decreased

Figure 6.8: Three-dimensional design of the mixer with three mixing units.

significantly by reducing channel width. Convection is still too large compared to diffusion. From a different point of view, reducing the channel width also means increasing the surface-to-volume ratio and thus increasing the contact surface of the mixing streams.

Based the above design considerations, we can utilize advection to produce the transversal flow, to improve mixing by increasing the contact surface, and at the same time to introduce chaotic advection into the flow. These considerations lead to the design of the following twist switching mixer.

Micromixer design

Due to small channel size in microfluidics, the Reynolds number is small. Therefore, the flow is laminar. The non-uniform velocity results in the so-called Taylor dispersion. Thus, if the contact surface and the other surface of the flow streams can be switched, non-uniformity can be improved. Furthermore, the contact surface can be increased and possible chaotic advection can be introduced. Figure 6.8 shows the schematics of the channel shape. The channel rotates the flow 90° every segments of the mixing unit. Figure 6.9 shows the expected flow switching effect in one mixing unit. The two flow will follow the shape change of the channel and twist in each other. Furthermore, due to the three-dimensional structure and the change of the channel cross section shape, chaotic advection occurs and enhances mixing.

Figure 6.9: Design principle schematics of the mixer in one unit.

In the previous works [82, 83, 84], the three-dimensional channel shapes were implemented through a complicated fabrication processes. The mixer in [82] was fabricated in silicon substrate through double side etching technique. This fabrication process is of high cost and long process time. The mixer in [83, 84] were fabricated in PDMS, which is popular in the past few years. However in small batch manufacturing or prototyping for research purpose, this technique has the same drawbacks of high cost and long process time, due to the fabrication of the replica master.

To minimize the fabrication time and cost, the three-dimensional channel shapes of our mixer was formed via the structures on two separate layers, Fig. 6.10. The planar structure was mirrored and fabricated on two sheets. Through alignment holes, the three-dimensional channel was obtained after bonding. This design can simplify the fabrication process significantly.

Fabrication

A rapid prototyping process with direct laser micromachining on polymer surface was used for the fabrication of the micromixer. Different from the previous work in Chapter 4, laser raster scanning was used to engrave channel with high aspect ratio and rectangular cross section on the surface of a 1 mm thick PMMA sheet. The aspect ratio can be adjusted by controlling laser power and moving speed. Due to the special channel structure, one of the designed two layers was mirrored in order to form the three-dimensional structure. Fig-

Figure 6.10: Schematics of implement of three-dimensional structure.

ure 6.11c and 6.11d show the structure on the bottom layer and the top layer, respectively. For the convenience of the further experiments, the position marks were also ablated on the surface. After laser engraving, the chips were rinsed in alcohol first, and then cleaned with DI-water in a ultrasonic cleaner for 15 minutes, and rinsed again using DI-water. After cleaning, the chips was dried.

The final structure was obtained by a customized bonding technique. First, the two layers of the chip were stacked together with contact of the engraved surfaces through three aligned holes and cross marks on the surface. The aligned rods were made of elastomer plastics. Their diameters were slightly larger than that of aligned holes and the rods have a shape of tapered cylinder, which is longer than aligned holes. This shape allows the rod to be inserted into the chips and also to obtain high precision alignment. After the alignment, the parts of the aligned rods outside chips were cut off. Bonding was carried out in a hotplate. The chips were heated to 165 °C and maintained for 30 minutes under pressure. Two polished silicon wafers were put between the two disks of the hotplate. For relieving thermal stress, annealing was carried out at 80 °C. This temperature was selected

Figure 6.11: Photos of the micromixers: (a) fabricated twist micromixer; (b) fabricated Y-mixer; (c) laser engraved bottom layer for twist micromixer; (d) laser engraved top layer for twist micromixer.

just below the glass transition temperature of PMMA (105°C). Figure 6.11a shows the bonded micromixer with fluidic interconnects. The bonding technique yields a good hermetical seal. There was no leakage in all characterized micromixers. The high quality bonding interface is shown in Fig. 6.12.

In the fabricated micromixer, six mixing units (Fig. 6.9) and one segment of straight channel as outlet duct were included. Due to the opaque characteristics of the laser engraved surface, the observation windows were selected as the first segment of each mixing unit. To compare the performance of the mixer, a Y-mixer with the same channel size was fabricated using the same method, Fig. 6.11b. The channel depth is $220\text{ }\mu\text{m}$; the average width is $820\text{ }\mu\text{m}$; and the channel length is 55 mm . The corresponding hydraulic diameter is $694\text{ }\mu\text{m}$.

6.2.2 Experiments

For characterization, the same method as described in Section 4.2.2 were used. The dye and DI-water were primed into the mixer by two precision syringes (Hamilton GasTight μl series, 1 ml). In the experiments, $4\times$ objective (NA 0.13) was used. The total size of the area of interest is $1584\text{ }\mu\text{m} \times 1188\text{ }\mu\text{m}$. To investigate the flow in the channel, the velocity field measurement was carried out with microPIV. $3\text{ }\mu\text{m}$ Duke red particles were selected for tracing the flow. Among the evaluation, the integration area is $32\text{ pixels} \times 32\text{ pixels}$

Figure 6.12: Micrographs of channel cross sections of the micromixers: (a) cross section of the horizontal channel; (b) cross section of the vertical channel.

with 50 % overlap ratio.

6.2.3 Results and discussions

The intensity images of six observation windows (Fig. 6.9) and outlet were captured along the channel. As reference, the same mixing conditions were applied to the Y-mixer. Figure 6.13 gives the comparison of the two mixers. From the results, at all flow rates, the twist mixer always had better performance than the Y-mixer. Furthermore, the switching flow can be observed at relatively higher flow rates, Fig 6.13b ($Pe=2493$, $Re=3.28$) and 6.13c ($Pe=7124$, $Re=9.37$). This phenomena can be seen clearly in the gray scale images, Fig. 6.14. In Fig. 6.14a and b, the high intensity is on the right side of the channel; in Fig. 6.14c, the interface is obscure. But in Fig. 6.14c and e, the high intensity is on the left side of the channel. The flow streams were switched. The flow pattern changes dramatically from one mixing unit to the next. Thus, the flow transition field in this area between the units are of interest. Figure 6.15 shows one of the vector plot of the flow field. The rapid flow transition can be seen clearly in the right bottom corner.

6.3 Summary

Two novel designs of micromixer were presented in this chapter. Both designs are passive mixers. In the first design, two-phase hydraulic focusing was used to narrow the

Figure 6.13: Comparison the normalized concentration of the twist mixer and normal Y-mixer: (a) flow rate $Q=200 \mu\text{l/h}$, $Pe=285$, $Re=0.375$; (b) flow rate $Q=1750 \mu\text{l/h}$, $Pe=2493$, $Re=3.28$; (c) flow rate $Q=5000 \mu\text{l/h}$, $Pe=7124$, $Re=9.37$.

Figure 6.14: Gray scale intensity images of six observation windows along the channel at flow rate of $1750 \mu\text{l/h}$ ($\text{Pe}=2493$, $\text{Re}=3.28$).

mixing flow width. Furthermore, the mixing flow width can be adjusted by changing viscosity ratio as well as flow rate ratio between the sheath flow and the mixing flow. In the second design, a three-dimensional twist-switching micromixer fabricated in a two-layer structure was presented and characterized. The experimental results showed excellent mixing performance. The twisting effects of the flow streams was also observed in the channel. Rapid flow transition was observed. The work offered a technique for low-cost fabrication of three-dimensional microfluidic devices.

Figure 6.15: Velocity vector plot for the third observation window at a flow rate of 1750 $\mu\text{l/h}$ ($\text{Pe}=2493$, $\text{Re}=3.28$).

Chapter 7

Conclusions and Outlook

7.1 Conclusions

This thesis focused on the mass transport and mixing effects in microscale and tried to establish both analytical and numerical models for better understanding of these effects. Works also have been done to explore new effects and parameters for mixing in microscale. Optimization parameters for parallel mixing in microscale were studied in this thesis. Furthermore, characterization was carried out to verify the theoretical works. Utilizing the knowledge from theoretical and experimental works, two novel designs were proposed and characterized.

The analytical models were based on laminar flow and mass conservation in convective-diffusive mixing. Two critical control equations were: Navier-Stokes equation and convective-diffusion equation in two-dimensional forms. Both of mixing with the same viscosity and different viscosities were considered. Due to the limitation of analytical method, numerical method was used for describing the concentration distribution in the case of the mixing with different viscosities. In the later model, Taylor dispersion was included. Besides 1:1 mixing ratio, the models can be extended to an arbitrary mixing ratio. Also, this solutions can be extended to parallel mixing in microscale. The velocity distribution and concentration of hydraulic focusing were modelled. In addition, the thesis discussed non-linear effects of mixing in microscale.

According to analytical model, Peclet number Pe was the most important parameters

for passive mixing. In parallel mixing, dimensionless mixing length κ is another important parameter. The two parameters were useful for designing and optimizing passive mixers.

To verify the theoretical models, a series of fully polymeric prototypes were fabricated. A rapid fabrication technique, direct laser micromachining, was adopted in prototyping combined with lamination technique. A direct thermal bonding technique was developed for a better seal between laminated layers. The experimental results show good agreements with theoretical data. In addition a quantitative method was offered to evaluate the performance of passive parallel micromixers.

Finally, two novel designs of micromixers were proposed. The first design was based on two-phase hydraulic focusing while the second design was based on twist switching in a three-dimensional channel. The characterization results proves the performance of these two designs.

7.2 Outlook

As mentioned in Chapter 2, the research on micromixers and mixing in microscale progressed quickly in the past three years. The importance of micromixer was recognized by academia and industry, but the development is still on its early stage. There are many problems that need to be solved and investigated. Many effects such as the roughness of the channel, electro-thermal coupled flow, multiple phase flow and thermal/pressure creep in mixing are still not investigated thoroughly. Furthermore, there is no standardized and mature quantitative method to evaluate the performance of micromixers.

Due to limitations of the analytical method and numerical methods, a combined method of analytical method with numerical method is a good choice for investigation of mixing in microscale. Many effects can be considered in such a model.

Passive micromixers work in relative low Reynolds number has the biggest potentials in the market of analytical analysis, bio assay and lab-on-a-chip. These micromixers have low sample consumption, stable operation and requires simple fabrication techniques.

Appendix A

Finite-Volume Method

A.1 Introduction

All convection-diffusion equations can be expressed by the general form:

$$\frac{\partial(\rho u_i \Phi)}{\partial x_i} = \frac{\partial}{\partial x_i} \left(\Gamma \frac{\partial \Phi}{\partial x_i} \right) + S \quad (\text{A.1})$$

where Φ denotes any dependent variables, Γ is the diffusion coefficient, u_i is the velocity at i ($i = x, y, z$) direction, and S is source term.

In the finite-volume method (FVM), the conservation principles are applied to a fixed region in space known as a control volume. The domain is divided into a number of control volume (CV) surrounding each grid point. The grid point is located at the center of the CV. All governing equation are integrated in each CV to derive an algebraic equation containing the grid point value of Φ . The discrete equation expresses the conservation principles in a finite control volume just as the partial differential equation express in an infinitesimal control volume. The resulting solution implied the conservation of quantities that is satisfied for any CV in the domain.

A.2 Discretized equation

A two-dimensional CV in Cartesian coordinate is shown in Fig. A.1. The CV interface is divided into four faces and differentiated by lowercase e, w, n, s corresponding to their direction to the central node P . The neighboring nodes are differentiated by uppercase

Figure A.1: A two-dimensional control volume in Cartesian coordinate.

E, W, N, S corresponding to their direction to the central node P . Separating $S = S_C + S_P\Phi_P$, the governing equation can be discretized as:

$$a_P\Phi_P = a_E\Phi_E + a_W\Phi_W + a_N\Phi_N + a_S\Phi_S + b \quad (\text{A.2})$$

with $a_E = \frac{\Gamma_e}{(\delta x)_e}\Delta y$, $a_W = \frac{\Gamma_w}{(\delta x)_w}\Delta y$, $a_N = \frac{\Gamma_n}{(\delta y)_n}\Delta x$, $a_S = \frac{\Gamma_s}{(\delta y)_s}\Delta x$, $b = S_C\Delta x\Delta y$ and $a_P = a_E + a_W + a_N + a_S - S_P\Delta x\Delta y$.

A.3 Solution Procedures

A two-dimensional numerical simulation programme is used in the thesis, Fig. A.2. The main programme (Fig. A.2a) is responsible to set up grids, define geometrical parameters, set initial value and boundary condition, read or write data file, assign the iteration value and calculate time, call subroutine, TIMITR, to get a convergent results in each step. Integer variables, ISTEP and ITER, represent the time iteration number and maximum iteration number in each step, respectively. The time step, DT, is assigned with a large value in steady state problem. The parameters of geometry are given or calculated and the initial values are set it SETUP1. The equation coefficients are established in SETUP2, where three routines, SOLVE, DIFLOW and GAMSOR are called. The SOLVE routine is called

Figure A.2: Flow chart of the numerical simulation programme.

to solve the equations. Tridiagonal matrix algorithm is used for solving process in SOLVE routine. In DIFLOW, the formulation of convection diffusion is calculated. The diffusion coefficient and source term are set in GAMSOR. Uniform grid is generated in UGRID, and results are printed in OUTPUT1. The calculation process is monitored by OUTPUT. The grid points and surfaces of CVs are generated in GRID. The density properties are set in DENS while boundary conditions are set in BOUND. UPDATE will refresh the data with new commotional results. GTIME can change the time step when necessary. The flow chart for illustrating the over-all scheme of the programme is shown in Fig. A.2.

A.4 Sensitivity of numerical simulation

The sensitivity of the grid was checked at the all cases. The method is to make the density larger. When the grid density was 2, 4, and 8 times of the original density, no difference was found when the data has three effective numbers. That is, the difference due to grid density is smaller than 0.1%. It is lower that the widely accepted number 1%.

A.5 Validation of the numerical simulation

To validate the numerical simulation method, a comparison between numerical simulation and analytical solution was carried out on the 1:1 mixing at the same channel with the same mesh grid and grid density. At numerical simulation, the Pe s were set to at the same as that in the analytical solution. They were 250, 500, and 750 respectively. The diffusion coefficient was set as $1.5 \times 10^{-9} \text{ m}^2/\text{s}$. The velocity were 375, 750, and 1125 $\mu\text{m}/\text{s}$. We found that the numerical diffusion exist. Epically at the end of the channel and near wall, this phenomena is larger. Therefore, at the three cases, we compared the values at the outlet and near wall (solution side), Fig. A.3. At small Pe , the largest difference was relative larger, but it is still small, only 1.5%. The two methods agree well with each other. It can be further used in the other similar simulation.

Figure A.3: Comparison between the numerical simulation and analytical solution: (a) normalized concentration at outlet; (b) normalized concentration near the wall (solution side).

Appendix B

Publication list

B.1 Journal papers

1. Z. Wu, T.Q. Truong, N. T. Nguyen, X. Huang: Characterization of Microfluidic Devices Using Micro Particle Image Velocimetry. *International Journal of Computational Engineering Science*, 4/3:269-272, 2003.
2. Zhigang Wu, N. T. Nguyen, X. Y. Huang. Non-linear diffusive mixing in microchannels: theory and experiments. *Journal of Micromechanics and Microengineering*, 14:604-611, 2004.
3. Z. Wu, N. T. Nguyen: Convective-diffusive transport in parallel lamination micromixers. *Microfluidics and Nanofluidics*, Springer, 2004, Vol. 7, No. 1, pp 13 - 20.
4. Z. Wu, N. T. Nguyen: Rapid mixing using two-phase hydraulic focusing in microchannels. *Biomedical Microdevices*, Springer, 2005, Vol. 7, No. 1, pp 13 - 20.
5. S. H. Chan, N. T. Nguyen, Z. Xia, Z. Wu: Development of a Polymeric Micro fuel cell containing laser-micromachined flow channels. *Journal of Micromechanics and Microengineering*, IOP, 2005, Vol. 15, No. 1, pp. 231-236.
6. Z. Wu, N. T. Nguyen: Hydrodynamic focusing in microchannels under consideration of diffusive dispersion: theories and experiments. *Sensors and Actuators B*, Elsevier, 2005, Vol. 107, No. 2, pp 965 - 974.

7. N. T. Nguyen, Z. Wu: Micromixers - a Review. *Journal of Micromechanics and Microengineering*, 2005, Vol. 15., No. 2, pp. R1-R16.
8. N. T. Nguyen, Z. G. Wu, X.Y. Huang, C.-Y. Wen: The Application of micro-PIV Technique in the Study of Magnetic Flows in a Micro-Channel. *Journal of Magnetism and Magnetic Materials*, Elsevier, 2005, Vol. 289, No. 3, pp 396 - 398.

B.2 Conference papers

1. N. T. Nguyen, X. Huang, Z. Wu. Flow Visualization for an In-Channel Microdispenser Using Micro Particle Image Velocimetry. In *The 7th Asian Symposium on Visualization*, Singapore, 2003,
2. T. Q. Truong, Z. Wu, N. T. Nguyen, X. Huang. Optimization and Validation of Rectification Microvalves with Micro Particle Image Velocimetry. In *The 7th Asian Symposium on Visualization*, Singapore, 2003,
3. N. T. Nguyen, T. Q. Truong, Z. Wu. A Piezoelectric Micropump Based on Polymeric Micromachining. In *microTAS2003*, Squaw Valley, California USA, 2003.
4. Z. G. Wu, N. T. Nguyen, Y. C. Kwok. Cross Micromixer: Model and Experiments. In *Asia-Pacific Conference of Transducers and Micro-Nano Technology APCOT MNT 2004*, Sapporo, Japan, 2004.
5. N. T. Nguyen, Z. G. Wu, Y. C. Kwok. Rapid Prototyping of Fully Polymeric Microfluidic Devices. In *Asia-Pacific Conference of Transducers and Micro-Nano Technology APCOT MNT 2004*, Sapporo, Japan, 2004.
6. Nguyen N.T., Wu Zhigang, 2004, Theoretical and Experimental Investigations of Hydrodynamic Focusing for Bio-MEMS Applications, First SBE International Conference on Bioengineering and Nanotechnology, Singapore, 2004.
7. N. T. Nguyen, Z. G. Wu, Y. C. Kwok. Modelling, Fabrication and Microfluidic Applications of Laser-machined Microchannels in PMMA. In *Asia-Pacific Conference*

of Transducers and Micro-Nano Technology APCOT MNT 2004, Sapporo, Japan, 2004.

B.3 Paper in proceedings

1. Z. Wu, N. T. Nguyen. A Dilute Mixing Model in Microchannels. In *Second MIT Conference on Computational Fluid and Solid Mechanics*, Boston, USA, 2003.

Bibliography

- [1] Nguyen N T and Wereley S T. *Fundamentals and Applications of Microfluidics*. Artech House, Boston, 2002.
- [2] Oosterbroek R E and van den Berg A. *Miniaturized System for Bio(Chemical) Analysis and Synthesis*. Elsevier, Amsterdam, 2003.
- [3] Klank H Geschke O and Telleman P. *Microsystem Engineering of Lab-on-a-Chip Devices*. John Wiley & Sons, New York, 2nd edition, 2004.
- [4] Madou M J. *Fundamentals of Microfabrication: The Science of Miniaturization*. CRC Press, Boca Raton, 2nd edition, 2002.
- [5] Gravesen P et al. Microfluidics - a review. *Journal of Micromechanics and Microengineering*, 3:168–192, 1993.
- [6] Elwenspoek M et al. Towards integrated microliquid handling systems. *Journal of Micromechanics and Microengineering*, 4:227–245, 1994.
- [7] Manz A et al. Miniaturized total chemical analysis systems: A novel concept for chemical sensing. *Sensors and Actuators B: Chemical*, 1:244–248, 1990.
- [8] Manz A et al. Electroosmotic pumping and electrophoretic separations for miniaturized chemical analysis systems. *Journal of Micromechanics and Microengineering*, 4:257–265, 1994.
- [9] Verpoorte E M J et al. Three-dimensional micro flow manifolds for miniaturized chemical analysis systems. *Journal of Micromechanics and Microengineering*, 4:246–256, 1994.

- [10] Sanders G H W and Manz A. Chip-based microsystems for genomic and proteomic analysis. *Trends in analytical chemistry*, 19:364–378, 2000.
- [11] Ho C M and Tai Y C. Micro-electro-mechanical-system (mems) and fluid flows. *Annual Review of Fluid mechanics*, 30:579–612, 1998.
- [12] Brody J P and Yager P. Diffusion-based extraction in a microfabricated device. *Sensors and Actuators A*, 58:13–18, 1997.
- [13] D H Fruman. Small is beautiful microhydrodynamics of yesterday, today and tomorrow. In *FLUIDOS-2001, VII International Seminar on Recent Advances in Fluid Mechanics, Physics of Fluids and Associated Complex Systems*, Buenos Aires, Argentina, 2001.
- [14] Kainiadakis G M and Beskok A. *Micro flows: fundamentals and simulation*. Springer-verlag, New York, 2002.
- [15] Huang X Y Nguyen N T and Toh K C. Memes–micropumps: a review. *ASME Transaction-Journal of Fluids Engineering*, 124:384–392, 2002.
- [16] Laser D J and Santiago J G. Memes–micropumps: a review. *Journal of Micromechanics and Microengineering*, 11:R35–R64, 2004.
- [17] Woias P. Micropumps-past, progress and future prospects. *Sensors and Actuators B*, page in print, 2004.
- [18] Bessoth F G Kakuta M and Manz A. Microfabricated devices for fluid mixing and their application for chemical synthesis. *Chemical Record*, 1:395–405, 2001.
- [19] Reyes D R et al. Micro total analysis systems. 1. introduction, theory, and technology. *Analytical Chemistry*, 74:2623–2636, 2002.
- [20] Auroux P A et al. Micro total analysis systems. 2. analytical standard operations and applications. *Analytical Chemistry*, 74:2637–2652, 2002.
- [21] Vilkner T et al. Micro total analysis systems. recent developments. *Analytical Chemistry*, 76:3373–3386, 2004.

- [22] Erbacher C et al. Towards integrated continuous-flow chemical reactors. *Mikrochimica Acta*, 131:19–24, 1999.
- [23] Einstein A. *Investigations on the Theory of the Brownian Movements*. Dover Publications, New York, 1956.
- [24] Ottino J M. Mixing, chaotic advection , and turbulence. *Annual review of fluid mechanics*, 22:207–253, 1990.
- [25] Brodsky R S. *Turbulence in Mixing Operations*. Academic Press Inc, New York, 1975.
- [26] Ottino J M. The mixing of fluids. *Scientific Ameican*, January:55–67, 1989.
- [27] Ottino J M. *The kinematics of mixing : stretching, chaos, and transport*. Cambridge University Press, New York, 1989.
- [28] Bird R B et al. *Transport phenomena*. John Wiley, 2nd edition.
- [29] et al. Hohreiter V. Size effects on turbulence and microfluidic mixing. In *Proceedings of United Engineetring Foundation Conference on Heat Transfer and Transport Phenomena in Microsystems*, Banff, Alberta, Canasda, 2000.
- [30] Cunningham R E and William R J J. *Diffusion in Gases and Porous Media*. Plenum Press, New York, 1980.
- [31] Ismagilov R F et al. Experimental and theoretical scaling laws for transverse diffusive broadening in two-phase laminar flows in microchannels. *Applied Physics Letters*, 76:2376–2378, 2000.
- [32] Kamholz A E and Yager P. Molecular diffusive scaling laws in pressure-driven microfluidic channels: deviation from one-dimensional einstein approximations. *Sensor and actuators B*, 82:117–121, 2002.
- [33] Song H et al. Experimental test of scaling of mixing by chaotic advection in droplets moving through microfluidic channels. *Applied Physics Letters*, 83:4664–4666, 2003.

- [34] Cussler E L. *Diffusion Mass Transfer in Fluid Systems*. Cambridge University Press, New York, 1984.
- [35] Kamholz A E et al. Quantitative analysis of molecular ineyactive in microfluidic channel: the t-sensor. *Analytical Chemistry*, 71:5340–5347, 1999.
- [36] Holden M A et al. Generating fixed concentration array in a microfluidic device. *Sensors and Actuators B*, 92:199–207, 2003.
- [37] Beard D A. Taylor dispersion of a solute in a microfluidic channel. *Journal of Applied Physics*, 89:4667–4669, 2001.
- [38] Dorfman K D and Brenner H. Comment on "taylor dispersion of a solute in a microfluidic channel". *Journal of Applied Physics*, 90:6553–6554, 2001.
- [39] Beard D A. Response to "comment on 'taylor dispersion of a solute in a microfluidic channel' ". *Journal of Applied Physics*, 90:6555–6556, 2001.
- [40] Brenner H and Edwards D A. *Macrotransport Processes*. Butterworth-Heinemann, 1993.
- [41] Handique K and Burns M A. Mathematical modeling of drop mixing in a slit-type microchannel. *Journal of Micromechanics and Microengineering*, 11:548–554, 2001.
- [42] Liepmann D Deshmukh A A and Pisano A P. Characterization of a micro-mixing, pumping, and valving system. In *Proceedings of Transducers '01, 11th International Conference on Solid-State Sensors and Actuators*, pages 779–782, Munich, Germany, 2001.
- [43] Josserand J Mengeaud V and Girault H H. Mixing processes in a zigzag microchannel: Finite element simulation and optical study. *Analytical Chemistry*, 74:4279–4286, 2002.
- [44] Park S J et al. Rapid three-dimensional passive rotation micromixer using the brackup process. *Journal of Micromechanics and Microengineering*, 14:6–14, 2004.

- [45] Wu Z Nguyen N T and Huang X Y. Non-linear diffusive mixing in microchannels: Theory and experiments. *Journal of Micromechanics and Microengineering*, 14:604–611, 2004.
- [46] Yi M and Bau H H. The kinematics of bend-induced mixing in micro-conduits. *International Journal of Heat and Fluid Flow*, 24:645–656, 2003.
- [47] Ward M C L Wong S H and Wharton C W. Micro t-mixer as a rapid mixing micromixer. *Sensors and Actuators B*, 100:365–385, 2004.
- [48] Lim D S W et al. Dynamic formation of ring-shaped patterns of colloidal particles in microfluidic systems. *Applied Physics Letters*, 83:1145–1147, 2003.
- [49] Böhm S et al. A rapid vortex micromixer for studying high-speed chemical reactions. In *Technical Proceedings of Micro Total Analysis Systems MicroTAS 2001*, pages 25–27, Monterey, CA, USA, 2001.
- [50] Wong S H et al. Investigation of mixing in a cross-shaped micromixer with static mixing elements for reaction kinetics studies. *Sensors and Actuators B*, 95:414–424, 2003.
- [51] Angeli P Gobby D and Gavriilidis A. Mixing characteristics of t-type microfluidic mixers. *Journal of Micromechanics and Microengineering*, 11:126–132, 2001.
- [52] Veenstra T T. Characterization method for a new diffusion mixer applicable in micro flow injection analysis systems. *Journal of Micromechanics and Microengineering*, 9:199–202, 1999.
- [53] Jackman R J et al. Microfluidic systems with on-line uv detection fabricated in photodefineable epoxy. *Journal of Micromechanics and Microengineering*, 11:263–269, 2001.
- [54] Möbius et al. A sensor controlled processes in chemical microreactors. In *Proceedings of Transducers '95, 8th International Conference on Solid-State Sensors and Actuators*, pages 775–778, Stockholm, Sweden, 1995.

- [55] Koch M et al. Improved characterization technique for micromixers. *Journal of Micromechanics and Microengineering*, 9:156–158, 1999.
- [56] Haverkamp V. et al. The potential of micromixers for contacting of disperse liquid phases. *Fresenius Journal of Analytical Chemistry*, 364:617–624, 1999.
- [57] de Mello A J Bessoth F G and Manz A. Microstructure for efficient continuous flow mixing. *Anal. Comm.*, 36:213–215, 1999.
- [58] Kakuta M et al. Micromixer-based time-resolved nmr: Applications to ubiquitin protein conformation. *Analytical Chemistry*, 76:956–960, 2003.
- [59] Fluri K et al. Integrated capillary electrophoresis devices with an efficient postcolumn reactor in planar quartz and glass chips. *Analytical Chemistry*, 68:4285–4290, 1996.
- [60] Hadd A G et al. Microchip device for performing enzyme assays. *Analytical Chemistry*, 69:3407–3412, 1996.
- [61] McKnight T E Jacobson S C and Ramsey J M. Microfluidic devices for electrokinetically driven parallel and serial mixing. *Analytical Chemistry*, 71:4455–4459, 1999.
- [62] Brody J P Knight J B, Vishwanath A and Austin R H. Hydrodynamic focusing on a silicon chip: Mixing nanoliters in microseconds. *Physical Review Letters*, 80:3863, 1998.
- [63] Jensen K. Chemical kinetics: Smaller, faster chemistry. *Nature*, 393:735–736, 1998.
- [64] Ozers M S Walker G M and Beebe D J. Cell infection within a microfluidic device using virus gradients. *Sensors and Actuators B*, 98:347–355, 2004.
- [65] Hinsmann P et al. Design, simulation and application of a new micromixing device for time resolved infrared spectroscopy of chemical reactions in solutions. *Lab on a Chip*, 1:16–21, 2001.

- [66] Branebjerg J et al. Fast mixing by lamination. In *Proceedings of MEMS'96, 9th IEEE International Workshop Micro Electromechanical System*, pages 441–446, San Diego, CA, 1996.
- [67] Schwesinger N et al. A modular microfluid system with an integrated micromixer. *Journal of Micromechanics and Microengineering*, 6:99–102, 1996.
- [68] Gray B L et al. Novel interconnection technologies for integrated microfluidic systems. *Sensors and Actuators A*, 77:57–65, 1999.
- [69] Yager P Munson M S. Simple quantitative optical method for monitoring the extent of mixing applied to a novel microfluidic mixer. *Analytica Chimica Acta*, 507:63–71, 2004.
- [70] He B et al. A picoliter-volume mixer for microfluidic analytical systems. *Analytical Chemistry*, 73:1942–1947, 2001.
- [71] Melin J et al. A fast passive and planar liquid sample micromixer. *Lab on a Chip*, 4:in press, 2004.
- [72] Miyake R et al. Micro mixer with fast diffusion. In *Proceedings of MEMS'93, 6th IEEE International Workshop Micro Electromechanical System*, pages 248–253, San Diego, CA, 1993.
- [73] Miyake R et al. A highly sensitive and small flow-type chemical analysis system with integrated absorbionmetric micro-flowcell. In *Proceedings of MEMS'97, 10th IEEE International Workshop Micro Electromechanical System*, pages 102–107, Nagoya, Japan, 1997.
- [74] Rong W Larsen U D and Telleman P. Design of rapid micromixers using cfd. In *Proceedings of Transducers '99, 10th International Conference on Solid-State Sensors and Actuators*, pages 200–203, Sendai, Japan, 1999.
- [75] Seidel R U et al. Capillary force mixing device as sampling module for chemical analysis. In *Proceedings of Transducers '99, 10th International Conference on Solid-State Sensors and Actuators*, pages 438–441, Sendai, Japan, 1999.

- [76] Gray M L Voldman J and Schmidt M A. An integrated liquid mixer/valve. *Journal of Microelectromechanical Systems*, 9:295–302, 2000.
- [77] Nguyen N T. *Mikrofluidik: Entwurf, Herstellung und Charakterisierung*. Teubner Verlag, Stuttgart, 2004.
- [78] Wang H et al. Numerical investigation of mixing in microchannels with patterned grooves. *Journal of Micromechanics and Microengineering*, 13:801–808, 2003.
- [79] Lin Y et al. Ultrafast microfluidic mixer and freeze-quenching device. *Analytical Chemistry*, 75:5381–5386, 2003.
- [80] Wang H et al. Optimizing layout of obstacles for enhanced mixing in microchannels. *Smart Materials and Structures*, 11:662–667, 2002.
- [81] Thomas O M Jones S W and Aref H. Chaotic advection by laminar flow in a twisted pipe. *Journal of Fluid Mechanics*, 209:335–357, 1989.
- [82] Liu R H et al. Passive mixing in a three-dimensional serpentine microchannel. *Journal of Microelectromechanical Systems*, 9:190–197, 2000.
- [83] Vijayendran et al. Evaluation of a three-dimensional micromixer in a surface-based biosensor. *Langmuir*, 19:1824–1828, 2003.
- [84] Chen H and Meiners J C. Topologic mixing on a microfluidic chip. *Applied Physics Letters*, 84:2193–2195, 2004.
- [85] Jen C P et al. Design and simulation of the micromixer with chaotic advection in twisted microchannels. *Lab on a Chip*, 3:77–81, 2003.
- [86] Liepmann D Evans J and Pisano A P. Planar laminar mixer. In *Proceedings of MEMS'97, 10th IEEE International Workshop Micro Electromechanical System*, pages 96–101, Nagoya, Japan, 1997.
- [87] Johnes S and Aref H. Chaotic advection in pulsed source-sink systems. *Physics of Fluids*, 31:469–485, 1998.

- [88] Ross D Johnson T J and Locascio L E. Rapid microfluidic mixing. *Analytical Chemistry*, 74:45–51, 2002.
- [89] Stroock A D et al. Chaotic mixer for microchannels. *Science*, 295:647–451, 2002.
- [90] Stroock A D and Whitesides G M. Controlling flows in microchannels with patterned surface charge and topography. *Accounts of Chemical Research*, 36:597–604, 2003.
- [91] Erickson D Biddiss E and Li D. Heterogeneous surface charge enhanced micromixing for electrokinetic flows. *Analytical Chemistry*, 76:3208–3213, 2004.
- [92] Hau W I et al. Surface-chemistry technology for microfluidics. *Journal Micromechanics and Microengineering*, 13:272–278, 2003.
- [93] Kim D S et al. A barrier embedded chaotic micromixer. *Journal Micromechanics and Microengineering*, 14:798–805, 2004.
- [94] Bertsch A et al. Static micromixers based on large-scale industrial mixer geometry. *Lab on a Chip*, 1:56–60, 2001.
- [95] Bertsch A et al. Laminar flow in static mixer with helical elements. *online at <http://www.bakker.org/cfmbook/lamstat.pdf>*, 1998.
- [96] Fujii T Hosokawa K and Endo I. Droplet-based nano/picoliter mixer using hydrophobic microcapillary vent. In *Proceedings of the IEEE International Workshop Micro Electromechanical System*, pages 388–393, Piscataway, NJ, USA, 1999.
- [97] Pamula V K Paik P and Fair R B. Rapid droplet mixers for digital microfluidic systems. *Lab on a Chip*, 3:253–259, 2003.
- [98] Lyon A D Tice J D and Ismagilov R. F. Effects of viscosity on droplet formation and mixing in microfluidic channels. *Analytica Chimica Acta*, 507:73–77, 2003.
- [99] Liepmann D Deshmukh A A and Pisano A P. Continuous micromixer with pulsatile micropumps. In *Technical Digest of the IEEE Solid State Sensor and Actuator Workshop*, pages 73–76, Hilton Head Island, SC, 2000.

- [100] Fujii et al. A plug and play microfluidic device. *Lab on a Chip*, 3:193–197, 2003.
- [101] Glasgow I and Aubry N. Enhancement of microfluidic mixing using time pulsing. *Lab on a Chip*, 3:114–120, 2003.
- [102] Niu X Z and Lee Y K. Efficient spatial-temporal chaotic mixing in microchannels. *Journal of Micromechanics and Microengineering*, 13:454–462, 2003.
- [103] Okkels F and Tabeling P. Spatiotemporal resonances in mixing of open viscous fluids. *Physical Review Letters*, 92:038301, 2004.
- [104] Ho C M Suzuki H. A magnetic force driven chaotic micro-mixer. In *Proceedings of MEMS'02, 15th IEEE International Workshop Micro Electromechanical System*, pages 40–43, Las Vegas, Nevada, 2002.
- [105] Ryu K S Lu L H and Liu C. A magnetic microstirrer and array for microfluidic mixing. *Journal of Microelectromechanical Systems*, 11:462–469, 2002.
- [106] Aubry N El Moutar A O and Batton J. Electro-hydrodynamic micro-fluidic mixer. *Lab on a Chip*, 3:273–280, 2003.
- [107] Tabeling P Deval J and Ho C M. A dielectrophoretic chaotic mixer. In *Proceedings of MEMS'02, 15th IEEE International Workshop Micro Electromechanical System*, pages 36–39, Las Vegas, Nevada, 2002.
- [108] Lee Y K et al. Chaotic mixing in electrokinetically and pressure driven micro flows. In *Proceedings of MEMS'01, 14th IEEE International Workshop Micro Electromechanical System*, pages 483–486, Interlaken, Switzerland, 2001.
- [109] Letteri G L et al. Consequences of opposing electrokinetically and pressure-induced flows in microchannels of varying geometries. In *Proc. Micro Total Analysis Systems*, pages 351–354, Enschede, NL, 2000.
- [110] Santiago J G Oddy M H and Mikkelsen J C. Electrokinetic instability micromixing. *Analytical Chemistry*, 73:5822–5832, 2001.

- [111] Tang Z et al. Electrokinetic flow control for composition modulation in a microchannel. *Journal of Micromechanics and Microengineering*, 12:870–877, 2002.
- [112] Yi M Bau H H, Zhong J. A minute magneto hydro dynamic (mhd) mixer. *Sensors and Actuators B*, 79:207–215, 2001.
- [113] White R M Moroney R M and Howe R T. Ultrasonically induced microtransport. In *Proceedings of MEMS'91, 3th IEEE International Workshop Micro Electromechanical System*, pages 277–282, Nara, Japan, 1991.
- [114] Zhu X and Kim E S. Acoustic-wave liquid mixer. In *Microelectromechanical Systems (MEMS) American Society of Mechanical Engineers, Dynamic Systems and Control Division (Publication) DSC. v 62*, pages 35–38, Fairfield, NJ, USA, 1997.
- [115] Rife J C et al. Miniature valveless ultrasonic pumps and mixers. *Sensors and Actuators A*, 86:135–140, 2000.
- [116] Zeng Y Vivek V and Kim E S. Novel acoustic-wave micromixer. In *Proceedings of MEMS'00, 13th IEEE International Workshop Micro Electromechanical System*, pages 668–673, Miyazaki, Japan, 2000.
- [117] Hauser K Woias P and Yacoub-George E. An active silicon micromixer for mtas applications. In *Micro Total Analysis Systems 2000*, pages 277–282, Boston, MA, USA, 2000.
- [118] Yasuda K. Non-destructive, non-contact handling method for biomaterials in micro-chamber by ultrasound. *Sensors and Actuators B*, 64:128–135, 2000.
- [119] Yang Z et al. Active micromixer for microfluidic systems using lead-zirconate-titanate(pzt)-generated ultrasonic vibration. *Electrophoresis*, 21:116–119, 2000.
- [120] Yang Z et al. Ultrasonic micromixer for microfluidic systems. *Sensors and Actuators B*, 93:266–272, 2001.
- [121] Liu R H et al. Bubble-induced acoustic micromixing. *Lab on a chip*, 2:151–157, 2002.

- [122] Liu R H et al. Hybridization enhancement using cavitation microstreaming. *Analytical Chemistry*, 75:1911–1917, 2003.
- [123] Yaralioglu G G et al. Ultrasonic mixing in microfluidic channels using integrated transducers. *Analytical Chemistry*, 76:3694–3698, 2004.
- [124] Yang T Mao H and Cremer P S. A microfluidic device with a linear temperature gradient for parallel and combinatorial measurements. *Journal of American Chemical Society*, 124:4432–4435, 2002.
- [125] Tsai J H and Lin L. Active microfluidic mixer and gas bubble filter driven by thermal bubble pump. *Sensors and Actuators A*, 97-98:665–671, 2002.
- [126] Schueller O Whitesides G Duffy D, McDonald J. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Analytical Chemistry*, 70:4974–4984, 1998.
- [127] Nguyen N T et al. Micro checkvalves for integration into polymeric microfluidic devices. *Journal of Micromechanics and Microengineering*, 14:69–75, 2004.
- [128] Nguyen N T et al. A polymeric microgripper with integrated thermal actuators. *Journal of Micromechanics and Microengineering*, 14:969–974, 2004.
- [129] Weigl B and Yager P. Microfluidic diffusion-based separation and detection. *Science*, 283:346–347, 1999.
- [130] Burke B J and Regnier F E. Stopped-flow enzyme assays on a chip using a micro-fabricated mixer. *Analytical Chemistry*, 75:1786–1791, 2003.
- [131] Dertinger S K W et al. Generation of gradients having complex shapes using microfluidic networks. *Analytical Chemistry*, 73:1240–1246, 2001.
- [132] Holden M A et al. Microfluidic diffusion diluter: Bulging of pdms microchannels under pressure-driven flow. *Journal of Micromechanics and Microengineering*, 13:412–418, 2003.
- [133] Yang M et al. Generation of concentration gradient by controlled flow distribution and diffusive mixing in a microfluidic chip. *Lab on a Chip*, 2:158–163, 2002.

- [134] Gaskell D R. *Introduction to Thermodynamics of Materials*. Taylor & Francis, London, 1995.
- [135] Bassi F A et al. An improved optical method of obtaining the diffusion coefficient from the refractive index gradient profile. *Journal of Physics E: Scientific Instruments*, 10:249–253, 1977.
- [136] Timmermans J. *The Physio-Chemical Constants of Binary Systems in Concentrated Solution*. Interscience, New York, 1960.
- [137] Darken L S. Diffusion, mobility and their interrelation through free energy in binary metallic systems. *Transactions of the American Institute of Mining and Metallurgical Engineers*, 175:184–201, 1948.
- [138] Vignes A. Diffusion in binary solutions. *Industrial and Engineering Chemistry Fundamentals*, 5:189, 1966.
- [139] Becker H and Gartner C. Polymer microfabrication methods for microfluidic analytical application. *Electrophoresis*, 21:12–26, 2000.
- [140] Soper S A et al. Polymetric microelectromechanical systems. *Analytical Chemistry*, 72:643A, 2000.
- [141] Becker H and Locascio L E. Polymer microfluidic devices: A review. *Talanta*, 56:267–287, 2002.
- [142] Cowie J M G. *Polymers: Chemistry and Physics of Modern Materials*. Chapman and Hall, New York, 2nd edition, 1991.
- [143] Srinivasan R E. Ablation of polymethyl methacrylate films by pilsed (ns) ultraviolet and infrad (9.17 μm) lasers: a comparative study by ultrafast imaging. *Journal of Applied Physics*, 73:2743–2750, 1993.
- [144] Klank H et al. CO₂-laser micromachining and back-end processing for rapid production of pmma-based microfluidic systems. *Lab on a chip*, 2:242–246, 2002.

- [145] Kashiwagi T et al. Effects of weak linkages on the thermal and oxidative degradation of poly(methyl methacrylates). *Macromolecules*, 19:2160–2168, 1986.
- [146] Nising P et al. On the degradation and stabilization of poly(methyl methacrylates) in a continuous process. *Chem. Eng. Technol.*, 26:599–604, 2003.
- [147] Alonso-Amigo M G and Adams T. Development of a plastic microfluidic chip. *IVD Technology*, 03:41, 2003.
- [148] Westerweel J. Fundamentals of digital particle image velocimetry. *Measurement science and technology*, 8:1379–1392, 1997.
- [149] Olsen M G and Adrian R J. Brownian motion and correlation in particle image velocimetry. *Optics and Laser Technology*, 32:621–627, 2000.
- [150] Reichman J. *Handbook of optical filters for fluorescence microscopy*. Chroma Technology Co., 2000.
- [151] Keane R D and Adrian R J. Theory of cross-correlation analysis of PIV images. *Appl. Sci. Res.*, 49:191–215, 1992.