MICROBUBBLE STREAMING FLOWS FOR NON-INVASIVE PARTICLE MANIPULATION AND LIQUID MIXING

BY

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DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Mechanical Engineering in the Graduate College of the University of Illinois at Urbana-Champaign, 2013

Urbana, Illinois

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Abstract

Liquid transport at small length scales plays an increasingly important role in many emerging and inter-disciplinary fields, such as micro/nano manufacturing processes, biomedical engineering, lab-on-a-chip diagnostic technology, micro fuel cell, and bio-fluidics. As the length scale shrinks, the synergy of micro-hydrodynamics with various external force fields (capillary forces, acoustic forces, electric and magnetic fields, and optical forces) has emerged as an exciting inter-disciplinary field. In this dissertation, we study an emerging actuating mechanism – microbubble steady streaming flows – by emphasizing both fundamental understanding and the design and applications of bubble-based microfluidic devices.

Under periodic acoustical driving, microbubbles with radius 20 – 100 µm absorbed or attached to a solid wall initiate steady streaming flows around the bubbles. These flows are driven by the Reynolds stresses in the boundary layer, a consequence of the nonlinearity of the Navier-Stokes equations. In the first part of the dissertation, we focus on manipulating micron-sized objects with microbubble streaming flows. We observe that in microbubble streaming flows, micro-particles (radius 1–5 µm) exhibit size-dependent behaviors: particles of different sizes follow different characteristic trajectories. Superimposing bubble streaming flow and a Poiseuille flow shapes the flow into regions of closed streamlines and open streamlines. The combined flow fields allow selective trapping of particles by size and subsequent releasing of the trapped particles. We explain these mechanisms and exploit them as a novel and general concept of manipulating microparticles. By integrating acoustically driven bubbles as active elements in various microfluidic devices, we further demonstrate specific applications including switching, sorting, focusing, and pre-concentrating of micro-particles.
Although microbubble streaming flows have received increasing attention in microfluidics and have been used widely in recent years, a fundamental understanding of microbubble streaming flow lags behind the experimental progress. In the second part, we study the frequency dependence of bubble streaming flows, and the correlation between the bubble dynamics and the streaming flow patterns. In contrast to steady streaming due to simple harmonic oscillations of a solid object, microbubbles exhibit frequency dependent and more complex shape modes. As the streaming flow patterns are caused by oscillations of microbubbles in contact with walls of the set-up, an understanding of the bubble dynamics is crucial. In this part of the study, we aim to bridge the gap between the physical understanding and the experimental observations of this complex phenomenon. With high-speed imaging, we experimentally characterize the oscillation modes and the frequency response spectrum of such bubbles, driven by a pressure variation resulting from ultrasound in the range of \(1 \text{ kHz} \lesssim f \lesssim 100 \text{ kHz}\). We find that (i) the appearance of streaming flow patterns is governed by the relative amplitudes of bubble surface modes (normalized by the volume response), (ii) distinct, robust resonance patterns occur independent of details of the set-up, and (iii) the experimental results compare well with the prediction of our asymptotic theory. With the bubble dynamics known from both experiment measurement and theoretical prediction, we also perform the calculation of streaming flows from the bubble dynamics using the first principle approach.

The fundamental understanding of frequency dependent streaming flows in turn can guide the design of practical microfluidic applications, such as various strategies of effective mixing on the micron scale. In the final part of this dissertation, we investigate two general classes of mixing strategies utilizing microbubble streaming flows: (a) modulating the acoustic driving pattern, such as the duty cycle and driving frequency, and (b) controlling the arrangement of microbubbles, such as the number, position, and orientation of the microbubbles. More specifically, modulating duty cycling will change the steady streaming flow directly by breaking it into unsteady flows, and thus achieve more effective mixing. Modulating driving
frequency $f$ can alter the directions of the resulting far field streaming flow. Strategically switching between drastically different flow patterns leads to improved mixing. On the other hand, when using multiple bubbles as actuating elements, the distance, position, and arrangement will affect the interaction between the streaming flows caused by each individual bubbles. Steady three dimensional flow can thus be achieved through proper arrangement and positioning of the microbubbles. Finally, we show that combining strategies of (a) and (b) can yield even better mixing. Through these strategies, we also demonstrate the flexibility of using microbubble streaming flows as both active and passive micro-mixers.
To my family
The completion of this dissertation would not have been possible without the help of many people. First and foremost, I wish to express my deepest gratitude to my advisor Professor Sascha Hilgenfeldt, for his continuous guidance, encouragement, and support through my doctoral study. Prof. Hilgenfeldt not only introduced me to a fascinating research topic to work on, but also provided enlightening advice and comments, and guided the direction of this thesis research. In the meantime, I also thank him for giving me great freedom to explore on my own and encouraging collaboration with other research groups, such as Professor Jimmy Hsia’s group.

I have enjoyed the interesting research work with Professor Hsia and Dr. Huan Li to study micro-droplets on micro-textured surface. Additionally, I would like to thank Professor Hsia on his advice and opportunity to work together.

I would also like to thank my doctoral committee members – Professor David Saintillan, Professor Taher Saif and Professor William O’Brien for their valuable comments and helpful suggestions. I am thankful to many excellent professors and teachers – Professor Vanka, Professor Freund, and Professor Ewoldt for the pleasant interactions and the wonderful learning experiences from their classes.

I wish to thank the current and past members of our research group: Shreyas Jalikop, Bhargav Rallabandi, Lin Guo, and the many undergraduate researchers for the fruitful discussion and collaboration over the last few years.

I am grateful to the staff in the Department of MechSE, especially Kathy Smith, Emily Lange, Jennifer Hayden, for their help and assistance on many administrative matters. I have
also learnt many useful micro-fabrication techniques and tips from staff at the Micro-Nano-
Mechanical Systems Cleanroom, especially Glennys Mensing, Kathleen Motsegood, Bruce
Flachsbart, and Michael Hansen. I would like to thank the staff of the MechSE workshop
and facility office: Bob, Cliff, Kyle, Keith, Gary, and Vance for providing help on making
parts for my research and teaching.

Working as a teaching assistant in the department has been a wonderful and valuable
experience to me, and I would like to thank the instructors and lab managers who helped
me in many ways in the last few years, especially Dr. Keng Hsu, Dr. Bruce Flachsbart, and
Dr. John Williams.

Many thanks also go to my friends – Yong Zeng, Pinggu Wu, Jiaxi Lu, Qi Zhang, Rui
Liu, Lingjun Meng, Huan Li, Binglu Ruan, Huan Hu, Xin Chai, Xian Wei, Donghai Gai,
Xuemeng Zhang, Chun Ge, Xin Tang, Ru Wang, Vivek Natarajan, who have made my life
at Champaign-Urbana more enjoyable and memorable.

Finally, I would like to thank my family for their constant love and support over many
years. I am indebted to my girlfriend for her support, encouragement, and unwavering belief
in me.
# Table of Contents

List of Tables ......................................................................................................................... xi

List of Figures ......................................................................................................................... xii

Chapter 1 Introduction ............................................................................................................. 1
  1.1 Micro/nanofluidics ........................................................................................................... 1
  1.2 Microbubbles as actuating elements for microfluidics ..................................................... 3
  1.3 Acoustically driven microbubbles and steady streaming flows ....................................... 4
  1.4 Organization of the dissertation .................................................................................... 7
  1.5 Key accomplishments .................................................................................................... 8

Chapter 2 Experiment .............................................................................................................. 11
  2.1 General fabrication techniques of microfluidic devices ................................................ 11
  2.2 Fabrication with soft-lithography .................................................................................. 12
    2.2.1 Basic process flow of soft-lithography .................................................................. 12
    2.2.2 Photomask ........................................................................................................... 13
    2.2.3 SU-8 mold ........................................................................................................... 14
    2.2.4 PDMS replication ............................................................................................... 16
    2.2.5 Assembly of microfluidic devices ....................................................................... 17
  2.3 Equipment and materials .............................................................................................. 19
  2.4 Visualization and data analysis ...................................................................................... 21
    2.4.1 Particle tracking and streak visualization ............................................................ 21
    2.4.2 Particle imaging velocimetry .............................................................................. 22
    2.4.3 Astigmatism particle tracking velocimetry ............................................................ 22
    2.4.4 Imaging bubble motion ....................................................................................... 23
    2.4.5 Characterization of liquid mixing ....................................................................... 25

Chapter 3 Manipulation of microparticles ............................................................................ 27
  3.1 Introduction ..................................................................................................................... 27
  3.2 Microfluidic device designs ........................................................................................... 29
  3.3 Microbubble streaming flows: experimental observation and singularity modeling ....... 29
  3.4 Manipulating particles ................................................................................................... 36
    3.4.1 Size dependent behavior in bubble streaming flow ........................................... 36
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4.2</td>
<td>Shaping flow domain and selective trapping of microparticle by size</td>
<td>37</td>
</tr>
<tr>
<td>3.4.3</td>
<td>Mechanism of trapping</td>
<td>39</td>
</tr>
<tr>
<td>3.4.4</td>
<td>Releasing of trapped particles</td>
<td>40</td>
</tr>
<tr>
<td>3.5</td>
<td>Applications</td>
<td>42</td>
</tr>
<tr>
<td>3.5.1</td>
<td>Preconcentrating/Filtering/Enrichment of microparticles</td>
<td>43</td>
</tr>
<tr>
<td>3.5.2</td>
<td>Switching and sorting of microparticles</td>
<td>45</td>
</tr>
<tr>
<td>3.5.3</td>
<td>Focusing of microparticles</td>
<td>46</td>
</tr>
<tr>
<td>3.5.4</td>
<td>Understanding of the focusing process</td>
<td>49</td>
</tr>
<tr>
<td>3.6</td>
<td>Conclusions</td>
<td>53</td>
</tr>
<tr>
<td>3.5</td>
<td>Applications</td>
<td>42</td>
</tr>
<tr>
<td>3.5.1</td>
<td>Preconcentrating/Filtering/Enrichment of microparticles</td>
<td>43</td>
</tr>
<tr>
<td>3.5.2</td>
<td>Switching and sorting of microparticles</td>
<td>45</td>
</tr>
<tr>
<td>3.5.3</td>
<td>Focusing of microparticles</td>
<td>46</td>
</tr>
<tr>
<td>3.5.4</td>
<td>Understanding of the focusing process</td>
<td>49</td>
</tr>
<tr>
<td>3.6</td>
<td>Conclusions</td>
<td>53</td>
</tr>
</tbody>
</table>

Chapter 4  Frequency dependent bubble dynamics and bubble streaming flows

4.1 Introduction
4.1.1 Acoustic manipulation of fluid
4.1.2 Acoustic bubble streaming
4.2 Materials and methods
4.2.1 Experiment set-up
4.2.2 Data analysis
4.3 Correlation between bubble dynamics and streaming flow patterns
4.3.1 Flow patterns at different driving frequency
4.3.2 Two dimensional character of bubble dynamics
4.3.3 Mode amplitude and phase
4.3.4 Relative streaming strength and flow patterns
4.4 Comparison with asymptotic theory
4.4.1 Bubble dynamics
4.4.2 Steady streaming flows
4.4.3 Comparison of flow patterns and velocity
4.5 Geometric effect on bubble dynamics and steady flow patterns
4.5.1 Bubble with different shape
4.5.2 Bubble of different aspect ratio
4.5.3 Bubble with different wall separation distance
4.5.4 Summary of geometric effects
4.6 Conclusions

Chapter 5  Mixing strategies with microbubble streaming flows

5.1 Introduction
5.1.1 Introduction to mixing and mixing measures
5.1.2 Microscale mixing
5.2 Experiment
5.2.1 Design and fabrication of micro-mixers
5.2.2 Equipment and materials
5.2.3 Data analysis
5.3 Results and discussion
5.3.1 Single bubble mixers
5.3.2 Multi-bubble mixers

ix
List of Tables

1.1 Several commonly used forces and their scaling with length $L$ [1–3]. . . . . . . 2

3.1 Predicted values of $d_{gap}$ (setup A, $f = 13.7$ kHz, $\overline{u}_p = 0.64$ mm/s) under different driving voltages $V$. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 41

3.2 Experimental mean position and standard deviation $\Delta z$ of the microparticle trajectory bundles. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 49
List of Figures

2.1 Schematic of the process flow of soft-lithography technique: (a) exposure of photo-resist SU-8 through a photomask, (b) SU-8 mold after development, (c) pouring and curing of PDMS onto SU-8 mold and (d) peeling and sealing of PDMS replica to a flat PDMS layer. .................................................. 13

2.2 Photographs of a fabricated fluidic device: (a) disassembled view of components, (b) assembly of two substrates. .......................................................... 18

2.3 Snapshot of a microbubble at several time series under the illumination of the microscope light. (a1)-(a3) show the growth of the bubble when the PDMS chamber is empty. (b1)-(b3) show well-controlled bubble size (with a slightly visible decrease) by filling water into the chamber. ................................. 19

2.4 Schematic of the experimental set-up. The microfluidic device is mounted securely onto a movable microscope stage. The syringe pump is used for infusing liquid into the fluidic channel. The piezoelectric transducer using signals from the function generator and amplifier induces driving pressure to the bubble. The high-speed camera captures videos or images through an inverted microscope objective lens. Images and videos are transferred to the computer for analysis. .......................................................... 20

2.5 Flow field visualization and particle tracking ($f = 16.8$ kHz): (a) streak image by superimposing 1000 successive images, (b) tracking of an individual particle, and (c) calculated total velocity from the particle as a function of distance from the bubble center. ............................................... 22

2.6 PIV measurement: (a) velocity across a rectangular channel with aspect ratio $H/D = 2.5$ and theoretical prediction [4]; (b) velocity vector and vorticity contour of microbubble streaming flow at $f = 14.4$ kHz. ....................... 23

2.7 Typical particle tracking with astigmatism particle tracking velocimetry (APTV). (a) volumetric particle trajectories in microbubble streaming flows, and (b) a projected view of particle trajectories in the $x$-$z$ plane. ....................... 24

2.8 Improving time resolution with stroboscopic technique: (a)-(d) four consecutive snapshots captured at 100,000 fps of an oscillating bubble driven at $f = 23.1$ kHz; (e) radius change at 45° with respect to the initially undisturbed bubble; (f) improved temporal resolution by restacking the data points into one period $T = 1/f$. ....................... 25
3.1 Perspective schematic of experiment set-up. Different microfluidic devices can be mounted on the same base (glass slide). Setup A: top-view schematic of an elementary setup with one bubble, one inlet (I) and one outlet (O); Setup B: an H-shaped device with one bubble with two inlets and two outlets; Setup C: straight channel with alternating bubbles on both sides.

3.2 Streaming flow from a semi-cylindrical microbubble: (a) streak image ($f = 16.8$ kHz) without superimposed Poiseuille flow; (b) streaming velocity scale $u_s$ at different driving voltages. The prefactor (from least square fit) of 0.83 is close to the dimensional-analysis expectation of 1.

3.3 Polar coordinate system of the 2D geometry used in the calculation of microbubble streaming flow. The plane considered here is in $x$-$z$, such that $x = r \cos \theta$ and $z = r \sin \theta$.

3.4 Streamlines of the general solution: (a) $\psi_s(r, \theta, 1)$, (b) $\psi_s(r, \theta, 2)$, (c) $\psi_s(r, \theta, 3)$, (d) $\psi_s(r, \theta, 4)$, (e) $\psi_s(r, \theta, 5)$. (f) with proper coefficients chosen, the truncated series solution (Eq. 3.9) that uses seven terms is able to account for the presence of the bubble.

3.5 Streamlines calculated from RNW singularity theory. The separation of the two walls is the same as that of experiment, $H = 6.25a$. (a) a single bubble between two walls; (b) with an imposed Poiseuille flow (right to left as indicated the arrow); (c) two bubbles attached on two separation walls; and (d) a two-bubble configuration with an imposed Poiseuille flow. The open streamlines are indicated by blue streamlines, and the closed streamlines are red.

3.6 Particle trajectories of large and small particles. (a) initial positions of two different-sized particles; (b), (c) resulting closed trajectories, showing that large particles stay closer to the bubble.

3.7 Flow field of combined bubble streaming and main-channel Poiseuille flow (Poiseuille flow from right to left): (a) bubble is not excited; (b) $s = 0.17$; (c) $s = 0.043$; (d) $s = 0.021$; (e) $s = 0.014$; (f) $s = 0.009$. In (d), a critical streamline (red line) separates the flow domain into two parts.

3.8 Trajectories of large ($a_p = 5 \mu m$) and small ($a_p = 2.5 \mu m$) particles at different $s$: (a) $s = 0.031$; (b) $s = 0.022$ ($f = 23.1$ kHz).

3.9 Mechanism of trapping: (a) streak image highlighting the hyperbolic point $P$ and critical streamline (setup A, $s \approx 0.04$); (b) schematic detail of the boxed region of (a).

3.10 Experimental trajectories of 5 $\mu$m particles under the driving voltages of Table 3.1, showing good agreement with the predicted trajectories.

3.11 Releasing of $a_p = 5 \mu m$ particles (setup A, $f = 23.8$ kHz, $s \approx 0.015$, Poiseuille flow from right to left): (a) snapshot of accumulated 5 $\mu$m particle cluster; (b) streak image showing narrow trajectories of 5 $\mu$m particles (red) after releasing; (c) close-up of five simulated particle trajectories near the bubble surface, with small random perturbations applied at all times. All particles are trapped, then released by crossing the critical streamline in the indicated escape region (dashed box).
3.12 Preconcentration of microparticles: (a) snapshot of the 5-bubble set-up. The traces show the number of particles per time detected downstream of the last of five bubbles. The duty cycles are given by \((\tau_{on}; \tau_{off})\) values of (b) \(10s;10s\); (c) \(20s;10s\); (d) \(30s;10s\)......................... 43

3.13 Switching of 5 \(\mu m\) particles (setup B with particles injected in inlet \(I_1\); \(f = 20.8 kHz\)): (a) bubble is not excited, all particles exit at \(O_1\); (b) bubble streaming at \(s \approx 0.024\) splits the particles evenly between \(O_1\) and \(O_2\); (c) stronger streaming \((s \approx 0.018)\) diverts all particles to \(O_2\); (d) sorting a mixture of 5 \(\mu m\) and 2.5 \(\mu m\) particles at \(s \approx 0.013\): 100% of large particles exit at \(O_2\), 90% of small particles at \(O_1\)......................... 46

3.14 Active switching of 5 \(\mu m\) particles with a three-outlets microfluidic devices, with increasing streaming flows (i.e. decreasing \(s\)) from (a) to (d)......................... 46

3.15 Focusing of microparticles \((a_p = 5 \mu m)\) in microchannel: (a) bubbles are not excited, \(s = \infty\); (b) \(s = 0.23\); (c) \(s = 0.092\); (d) \(s = 0.066\); (e) \(s = 0.051\); (f) \(s = 0.043\); (g) \(s = 0.029\)......................... 48

3.16 Simultaneous visualization of flow field and large particle trajectories with a two bubble configuration with increasing streaming strength: (a) \(s \approx 0.24\); (b) \(s \approx 0.16\); (c) \(s \approx 0.11\); (d) \(s \approx 0.04\). The figures on the right panel show the corresponding particle \((a_p = 5 \mu m)\) trajectories......................... 50

3.17 Snapshot of microparticle focusing inside a multi-bubble straight channel at \(s \approx 0.05\): (a) experiment and (b) simulation......................... 51

3.18 Comparison of the particle focusing as a function of \(s\) between experiments and simulations for \(a_p = 5 \mu m, H = 250 \mu m\) and \(a = 40 \mu m\). Both experiments and simulations show a finite value of \(s \approx 0.05\) for optimum focusing......................... 53

4.1 Schematic of the experimental set-up for bubble dynamics measurement (not to scale): (a) and (b) show the side view and top view; (c) a perspective view of the semi-cylindrical bubble; (d) a snapshot of the undisturbed bubble (scale bar is 50 \(\mu m\)); (e) the coordinate system used to measure the bubble shape......................... 59

4.2 Bubble streaming flow patterns at different driving frequencies, with arrows indicating the orientations of the vortices. Outline of oscillatory bubble superposed over one cycle at different frequencies (e) 9.6 kHz, (f) 20.6 kHz, (g) 48.6 kHz and (h) 100.3 kHz......................... 61

4.3 Streak images at different depth \(y\) show similar streaming flow patterns \((f = 16.8 kHz)\). (a) middle of the bubble, \(y = 0\), (b) \(y = 10 \mu m\), (c) \(y = 20 \mu m\), (d) \(y = 30 \mu m\), (e) \(y = 40 \mu m\), and (f) near the top wall \(y = 50 \mu m\)......................... 63

4.4 (a) Streaming velocity \(\bar{u}_s \equiv u_s(y)/u_s(y = 0)\) and (b) oscillating amplitude \(\bar{\epsilon} \equiv \epsilon(y)/\epsilon(y = 0)\) at different depth \(y\)......................... 63

4.5 Measured mode amplitude \(a_n\) (a) and (b), and phase which is represented in terms of \(\sin(\phi_n - \phi_0)\) (c) and (d). Open symbols are for the glass substrate, filled symbols for the polystyrene substrate......................... 65

4.6 Normalized mode amplitudes \(\bar{a}_n\) with respect to monopole (a); and relative streaming strength \(I_n\) (b). Open symbols are for the glass substrate, filled symbols for the polystyrene substrate......................... 67
4.7 Percentage of power contained in each mode. The measurement is from a glass substrate device. 68

4.8 Streaming flow patterns with small tracer particles and better spatial resolution: (a) 14.4 kHz, (b) 30.9 kHz, (c) 48.4 kHz, and (d) 93.4 kHz (note small vortices between the dashed line and the bubble surface). 69

4.9 (a) Relative amplitudes $\bar{a}_n$ and (b) sine of phase angles $\phi_n$ of the first three even surface modes. The markers correspond to experimentally measured values for a nearly semi-cylindrical bubble, and the solid lines are predicted by the theory. The dotted lines in (a) indicate the undamped resonance frequencies of corresponding surface modes of free cylindrical bubbles. 71

4.10 Comparison of fountain flows in experiment and theory. (a) Experimental streamlines of Lagrangian steady flow at $f = 26.7$ kHz; (b) computed streaming pattern at the corresponding dimensionless frequency $\lambda = 9.32$, using amplitudes and phases from the analysis of [5]. The agreement is representative of the entire regime of fountain flow patterns. (c) Steady Lagrangian azimuthal velocity $v$ along lines of zero radial velocity (indicated as dot-dashed line in (b)), as a function of radial distance $r$: direct measurements from an experimental run at $f = 26.7$ kHz (○), computed from bubble oscillation amplitudes obtained from interface tracking experiments [5] of a different run at the same $f$ (—), and computed from theoretical bubble oscillation amplitudes using only $\lambda = 9.32$ as input (——). 75

4.11 Comparison of anti-fountain flow pattern from experiment and theory at high frequency, $\lambda = 30$ (i.e. $f = 96$ kHz). Note that the long axis of the vortex structures appears to point towards the “corners” where the bubble meets the wall. 76

4.12 Protruding bubble of different shapes. (a) standoff distance $\xi_a$ is measured between the horizontal wall and the center of a fitted circle of the bubble outline. (b1) to (b4) show four bubble shapes with different $\xi_a$. (c1) and (c2), (d1) and (d2) show the normalized amplitude $\bar{a}_n$ and the streaming intensity $I_n$ corresponding to the two extreme cases (b1) and (b4) respectively. 79

4.13 Resonance frequencies of $\bar{a}_n$ for different bubble sizes ($\xi$) that protrude from the same side channel, ($f_1$, $f_2$, and $f_3$ are the resonant frequency of $\bar{a}_1$, $\bar{a}_2$, and $\bar{a}_3$ respectively). 80

4.14 Streak images of microbubbles of different shapes (i.e. $\xi$) at the same driving frequency 26.7 kHz. Note that for the shape closer to semi-cylindrical shape, complex flow patterns due to higher order surface modes appear near the bubble pole. 80

4.15 Effect of different bubble depth $D$ on normalized mode amplitudes $\bar{a}_n$ and relative streaming strength $I_n$: (a) and (b) for a bubble depth $D = 100$ µm, and (c) and (d) for a bubble depth $D = 50$ µm. 82

4.16 Effect of wall separation distance $H$ on normalized mode amplitudes $\bar{a}_n$ and relative streaming strength $I_n$: (a) and (b) for wall separation $H = 1000$ µm, and (c) and (d) for wall separation $H = 250$ µm. 83
4.17 Steady streaming flow patterns with different wall separation distance $H$. (a1) and (a2) show the streaks at 16 kHz and 75 kHz inside a narrow microchannel ($H = 250 \mu m$). (b1) and (b2) show the streaks at 16 kHz and 75 kHz inside a wide microchannel ($H = 1000 \mu m$).

5.1 Schematic of a general mixing process. (a) initial distribution of white (solute, $c = 1$) and black (no solute, $c = 0$) in a domain. Striation thickness ($l_s$) is half of the white and black striation period. (b) reduced striation thickness through stirring (e.g. through chaotic advection, turbulence), with negligible diffusion. (c) molecule diffusion takes place and reduces the concentration gradients over striations.

5.2 (a) Schematic of the experimental set-up of mixing. And example mixer designs: (b) single side bubble mixer, (c) multiple side bubble mixer, (d) multiple top bubble mixer, (e) slanted top bubble mixer, (f) 3D arranged side and top bubble mixer, and (g) 3D arranged side and slanted top bubble mixer.

5.3 Schematic of two liquid streams flowing through the gap. (a) Bubble streaming flow superimposed with a Poiseuille flow creates one upstream and one downstream closed-loop vortices. Two liquids entering the main channel are funneled through the gap between the bubble surface and the upstream vortex. (b) Zoomed view near the bubble surface shows the reduced diffusion distance $\approx d_{gap}/2$ from the original channel width $H/2$.

5.4 Mixing in microstreaming flows combining a steady channel flow (right to left) with bubble streaming at low driving frequency ($f = 27.1$ kHz) (a) bubble is not excited; (b) bubble is driven at Voltage = 40 V; (c) bubble is driven at Voltage = 70 V; (d) bubble is driven at Voltage = 90 V; (e) coefficient of variation of the grayscale signal, $\sigma(c)/\bar{c}$; (f) mixing variance, $\phi^2$, calculated based on the sampling window indicated as a square box in (a), at different driving voltage. For both measures used here, smaller values indicate better mixing. Also note that the scale is log in (f), but linear in (e).

5.5 The modulus of the Fourier coefficients $c_k$ of the first 24 wave vectors. The flow condition is at low driving frequency ($f = 27.1$ kHz): (a) duty cycle interval $\tau = 5$ ms; (b) duty cycle interval $\tau = 20$ ms; (c) duty cycle interval $\tau = 50$ ms; (d) duty cycle interval $\tau = 200$ ms; (e) mixing variance, $\phi^2$, for different duty cycle intervals.

5.6 Mixing in microstreaming flows combining a steady channel flow (right to left) with duty cycling modulated bubble streaming at low driving frequency ($f = 27.1$ kHz) (a) duty cycle interval $\tau = 5$ ms; (b) duty cycle interval $\tau = 20$ ms; (c) duty cycle interval $\tau = 50$ ms; (d) duty cycle interval $\tau = 200$ ms.

5.7 The modulus of the Fourier coefficients $c_k$ of the first 24 wave vectors. The flow condition is at low driving frequency ($f = 27.1$ kHz): (a) duty cycle interval $\tau = 5$ ms; (b) duty cycle interval $\tau = 20$ ms; (c) duty cycle interval $\tau = 50$ ms; (d) duty cycle interval $\tau = 200$ ms.
5.8 Mean signal $\bar{c}$ of the sampling window for duty cycling modulated bubble streaming at low driving frequency ($f = 27.1$ kHz). (a) $\tau = 0$; (b) $\tau = 10$ ms; (c) $\tau = 20$ ms; (d) $\tau = 50$ ms; (e) $\tau = 100$ ms; (f) $\tau = 200$ ms. 104

5.9 Mixing in microstreaming flows combining a steady channel flow (right to left) with frequency modulated bubble streaming at low driving frequency ($f_l = 27.1$ kHz) and high frequency ($f_h = 91.3$ kHz) for different switching intervals. (a) $\tau = 5$ ms; (b) $\tau = 20$ ms; (c) $\tau = 50$ ms; (d) $\tau = 200$ ms; (e) mixing variance, $\phi^2$, at different switching interval. 105

5.10 A multiple side bubble micro-mixer driven continuously at low driving frequency ($f_l = 27.1$ kHz): (a) Voltage = 50 V; (b) Voltage = 60 V; (c) Voltage = 70 V; (d) Voltage = 80 V; (e) mixing variance, $\phi^2$, at different driving voltage. 108

5.11 Multiple top bubble micro-mixer driven continuously at low driving frequency ($f_l = 28.9$ kHz): (a1) Voltage = 50 V; (a2) Voltage = 70 V; (a3) Voltage = 90 V; (a4) mixing variance, $\phi^2$ for parallel top microbubbles. (b1)–(b4) are for the multi slanted top microbubble mixer. 109

5.12 Top view of a top microbubble. (a) optical microphotograph indicates gaps between the top bubble and the main channel wall, and the resulting round ends of the bubble. (b) trajectories of two incoming particles: a particle near the channel wall exhibits a complex 3D trajectory; while a particle coming along the center of the main channel stays in the $x$-$y$ plane. 110

5.13 Comparison of three micro-mixers driven continuously at low driving frequency ($f = 28.9$ kHz): (a1) 2D multi-bubble mixer; (b1) 3D multi-bubble mixer with one top and one side bubble alternatively arranged; (c1) 3D multi-bubble mixer with one top bubble and a pair of side bubbles alternatively arranged; (a2)–(b2) are the mixing variances at different driving voltage for the three mixers respectively. The small sketches show the design of the three mixers. 111

5.14 Combined strategy of mixing with a 2D bubble micro-mixer: (a) with duty cycling of different interval $\tau$ and (b) with switching between low $f$ and high $f$ of different interval $\tau$. 112

5.15 Combined strategy of mixing with a 3D bubble micro-mixer: (a) with duty cycling of different interval $\tau$ and (b) with switching between low $f$ and high $f$ of different interval $\tau$. 113

xvii
6.1 Concept of a continuous size filtering device with two inlets and two outlets: (a) two syringe pumps inject and withdraw liquid simultaneously with flow rate $Q_1$ between A and B and $Q_2$ between C and D. The streaming flows from an ultrasound driven bubble modulate the separatrix between the A-B and C-D flows. The gap between the bubble interface and the separatrix sets the critical size whether a particle is filtered. Particles with radius larger than the gap are kicked across the separatrix and transported to D. Smaller particles continue to flow towards B. The streak visualization of the separatrix are shown when the bubble is not excited (b) and when bubble is driven by the ultrasound (c). Switching of microparticles ($a_p = 5 \mu m$) to two different outlet ports are demonstrated in (d) and (e). In the experiment, the flow rate ratio between the two flows is $Q_2/Q_1 = 10$.

6.2 (a) Schematic of multiple connected devices for iterative size fractionation. By controlling the flow rate through the side channels, $Q_2$ and $Q'_2$, two different gap sizes are set, enabling filtering particles of different size in a continuous manner. (b) Principle of filtering by stiffness or deformability of the transported particles. A mixture of stiff (dark gray) and soft (light gray) biological objects of equal size are introduced. The latter deform sufficiently in the elongational shear flow in the gap to squeeze through to B, while the former cross the separatrix and are collected at D. (c) Coils of macromolecules (e.g. $\lambda$-DNA) are introduced at A. The relaxation time of one molecular species (red, spherical) is too small to undergo a coil-stretch transition and crosses the separatrix, while that of the other (magenta, elongated) results in a Weissenberg number above critical. (d) Giant unilamellar and multilamellar lipid vesicles grown by electroformation. (e) Trajectories of vesicles in the streaming flow of a single bubble (preliminary data).

6.3 Microbubble streaming flow patterns with angled walls at different driving frequency. In the top row, the walls have an angle of 15°: (a) 34.8 kHz, (b) 80.8 kHz, (c) 102.8 kHz (d) 141.8 kHz. In the bottom row, the walls have an angle of 30°: (e) 34.8 kHz, (f) 80.8 kHz, (g) 102.8 kHz (h) 141.8 kHz.

6.4 Switching and sorting at high frequency, $f = 116.3$ kHz. (a) the bubble attached to a straight wall does not switch or sort particles. (b) the bubble attached to an angled wall maintains the “fountain” loops, allowing switching and sorting of microparticles.

6.5 Liquid transport with microbubble streaming flows. (a) schematic of a closed loop fluidic device, with bubble-bump pairs along one portion of the device. (b1) a snapshot of two bubble-bump pairs; (b2) streaks visualization of the flow field. (c) net flow (right to left) is observed from a window located on another portion of the device.

A.1 Schematic of contact angle on various surfaces. (a) Young’s angle on a smooth solid surface; (b) Wenzel model and contact angle – liquid wetting the entire rough surface; (c) Cassie-Baxter model and contact angle – liquid suspending on top of the rough surface.
A.2 Schematic of the experiment setup for contact line visualization. The droplet with fluorescein is excited by the laser from the microscope objective. The microscope objective will detect the light emitted from the droplet and the image is constructed. .......................................................... 131

A.3 Fluorescence images of the contact area for a 20 µL droplet on top of PDMS cylindrical micropillars with a diameter of 57.1 µm and center-to-center spacing 138.4 µm. Pillars are in a square lattice. (a) droplet is in the Cassie-Baxter state; (b) droplet is in the Wenzel state. .......................................................... 132

A.4 Schematic of the apparatus used to measure the friction force of a liquid drop sliding on a micropatterned surface. In the experiment, the drop adheres to the PDMS sphere by wetting, which is then connected to a force sensor. The microscope stage, controlled by computer, is moving away from the sensor at a speed of V. The drop will then slide on the surface while remaining in contact with the stationary PDMS sphere. The friction force is measured by the force sensor and collected with a data acquisition system. The motion of the CL and contact area are captured by a high speed camera (Phantom v310, Vision Research) using a fluorescence microscope. .......................................................... 134

A.5 Close up image of the force sensor tip, on which a PDMS sphere is glued. (a) top view, and (b) side view. .......................................................... 134

A.6 Contact area of a 7 µL droplet wetting micropatterned surfaces with micropillars in square lattices. .......................................................... 136

A.7 Contact area of a 20 µL droplet wetting micropatterned surfaces with micropillars in square lattices. .......................................................... 137

A.8 CL motion of two 7 µL droplets coalescing on the micropatterned surface with pillar diameter 43.7 µm and φ = 0.16. .......................................................... 139

A.9 Measured force vs. displacement of the droplet as the droplet is dragged on a micropatterned surface. The surface consists of square pillars of side length 57.1 µm in a square lattice with center-to-center spacing of 134.8 µm. The initial slope of the force curve, k ≈ 102.9 mN/m, can be interpreted as the linear elasticity of a deforming droplet. In the inset, k_s ≈ 58.9 mN/m is the elasticity and W ≈ 0.187 × 10^{-9} J is the work required by moving the droplet over the spacing distance between the pillars. .......................................................... 140

A.10 Bottom view of a 10 µL droplet being dragged to slide on a PDMS substrate, composed of square pillars of side length 57.1 µm in a square lattice with center-to-center spacing of 134.8 µm. (a) PDMS sphere on the sensor probe just touching the droplet; (b) maximum force generated during sliding. The contact area between the droplet and substrate is at the maximum. The lateral sides of the CL (top and bottom in the figure) will shrink towards the center; (c) steady state sliding of the droplet. The force generated during sliding maintains an almost fixed amplitude and period due to regular pinning and depinning of the CL at the trailing edge. The shape of the contact area remains unchanged with successive jumps of the CL at the trailing edge and migration of the CL at the leading edge. .......................................................... 141
A.11 Side view of a 10 μL droplet being dragged on a PDMS substrate, composed of square pillars of side length 57.1 μm in a square lattice with center-to-center spacing of 134.8 μm. (a) PDMS sphere on the sensor probe just touching the droplet; (b) maximum deformation of the droplet, corresponding to the maximum force generated during sliding; (c) steady state sliding of the droplet. The force generated during sliding remains almost constant. The shape of the droplet remains unchanged, with successive detachment and attachment at the droplet’s trailing edge, and continuous wetting at the advancing edge.
Chapter 1

Introduction

1.1 Micro/nanofluidics

Micro/nanofluidics refers to the understanding, control, and manipulation of fluid flows at length scales of 10 nm to 1 mm [1–3]. Originated as a branch of Micro-Electromechanical-Systems (MEMS), micro/nanofluidics has developed rapidly over the last two decades and plays an increasingly important role in many engineering and science disciplines [1]. It has a wide range of applications, spanning from micro/nano manufacturing processes [6, 7] and biomedical engineering (lab-on-a-chip or micro Total Analysis Systems technology) [8] and portable power sources (micro fuel cells) [9] to bio-fluidics (swimming bacteria) [10, 11].

At these length scales (10 nm – 1 mm), from the physics point of view, micro/nanofluidics is no different, because the continuum hypothesis still holds and the classical physical laws are still valid [1–3]. However, at these smaller length scales, many different forces can become comparable or even significantly dominant compared to inertia. Several commonly encountered forces and their scaling with length are listed in Table 1.1. Taking surface tension force as an example, the ratio between surface tension force and gravitational force scales like $L^{-2}$. When $L$ goes smaller, surface tension dominates, and thus allows small insects such as water strider to walk on water easily. The shrinking length thus opens up new avenues and opportunities for us to control fluid flows with many other external fields, such as electric, magnetic, acoustic, and optical forces. The fusion of micro-hydrodynamics with the action of these external agents has emerged as many new exciting interdisciplinary fields: acoustofluidics [12], optofluidics [13], micro-magnetofluidics [14] etc.
Table 1.1: Several commonly used forces and their scaling with length $L$ [1–3].

<table>
<thead>
<tr>
<th>Type of force</th>
<th>Scaling with length $L$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravity</td>
<td>$\sim L^3$</td>
</tr>
<tr>
<td>Inertia</td>
<td>$\sim L^3$</td>
</tr>
<tr>
<td>Stokes drag</td>
<td>$\sim L$</td>
</tr>
<tr>
<td>Surface tension</td>
<td>$\sim L$</td>
</tr>
<tr>
<td>Dielectrophoresis</td>
<td>$\sim L^3$</td>
</tr>
<tr>
<td>Electrostatic</td>
<td>$\sim L^2$</td>
</tr>
<tr>
<td>Acoustic radiation</td>
<td>$\sim L^3$</td>
</tr>
</tbody>
</table>

Micro/nanofluidics is both a science and a technology, concerning not only the fundamental understanding of flow physics, but also the practical control, design and fabrication of fluidic devices [15]. There are excellent reviews focusing on the physics and fundamentals of fluid flow [16, 17] at small length scales, reviews covering the individual functions or components such as micromixer [18], micropumps [19], separation and sorting of particles/cells [20] as well as reviews with emphasis on specific biological or chemical applications [21, 22].

Micro/nanofluidics is in particular an enabling technology for one rapid developing interdisciplinary field – lab on a chip (LOC) or micro Total Analysis Systems ($\mu$TAS) [23–25]. The two main concepts of $\mu$TAS or LOC are miniaturization and integration. Conventional laboratory scale chemical and biochemical processes are shrunk onto inch-sized chips, through modern micro/nano fabrication techniques. These chemical and biological processes often take place in micro/nanoscale channels built on these chips. Many functions can be integrated onto a single chip: such as sample preparation purification, chemical reactions, and detection. These processes can be integrated and streamlined, to allow high degree of automation [26]. The advantages of miniaturization and integration are many: significant reduced consumption of chemical samples, increased efficiency, and reduced labor cost. LOC technology is an inter-disciplinary field that has found increasing applications, in chemistry, bio-chemistry and biology. The handling and manipulation of small fluid volumes in these micro/nano-scale geometries becomes essential for the success of $\mu$TAS technology, because chemical/biological samples often contain liquid or they are in the form of liquids.
1.2 Microbubbles as actuating elements for microfluidics

Unintentional bubbles are often undesirable in microfluidic environments [27, 28], because they can cause blockage of microchannel network, unequal flow distributions, or the failure of electric field based actuation. However, well controlled microbubbles can provide many valuable functions. One such example is medical microbubbles with radii of a few micrometers, which have been used to enhance ultrasound contrast for decades [29]. And more recently they have been used for therapeutic applications, such as drug delivery and gene transfection [30–32].

Microbubbles can be formed in many different ways, either actively or passively. A simple and passive approach is to create indentations/blind channels in a hydrophobic substrate, which will form protruding air pockets spontaneously when submerged into liquid [33–43]. These air pockets resemble 3D hemispherical bubbles [33–38], or 2D semi-cylindrical bubbles/membranes [39–43]. Another direct way to form bubbles is to inject air streams and liquid streams into the same channel (co-flow microfluidic devices) [44–46]. By adjusting the flow rate ratio between different phases and controlling the nozzle geometry, different flow regimes, such as bubbly, slug, and annular flows are possible [44]. In addition to the passive means of forming bubbles, there are a variety of active ways to form microbubbles, such as thermal heating [47–49], electrochemical [50–52] or electrolysis [53], and laser-induced cavitation [54, 55].

In microfluidics, well controlled microbubbles have found increasing applications as powerful actuators for liquid pumping, switching and valving, mixing enhancement, and manipulation of micro-objects. Cyclic growth and collapse of thermal bubbles in a nozzle-diffuser cause different liquid flow volumes out and into the chamber and result in net pumping [48, 56]. Direct formation of bubbles inside a microchannel is one effective and straightforward way of regulating the liquid flow [57, 58]. Hua et al. demonstrated electrochemical bubble valves by
integrating Platinum electrode pairs along a straight channel [57]. Microbubble valves and pumps can be used to perturb the laminar and steady flows for mixing enhancement [49]. The general advantages of using microbubbles include simple design and fabrication, no moving parts, and ease of integration. In addition, the small dimension favors the growth and dissolution of microbubbles. The rapid volumetric change can also provide larger displacement compared to piezoelectric membrane deflection. Additionally, much lower voltage can be used as compared to the higher voltage required for electrokinetic flows.

1.3 Acoustically driven microbubbles and steady streaming flows

When used in conjunction with acoustics, microbubbles serve as an agent to convert acoustic energy into hydrodynamic flows. Due to its compressibility, microbubble exhibits periodic volume expansion and contraction when exposed to an acoustic pressure field. Acoustic energies and forces can thus be focused to smaller length scales through these tiny vibrating bubbles. The dynamics of oscillating microbubbles is a classical topic in fluid mechanics [59]. The microbubble behaves differently depending on the acoustical driving amplitude. At one extreme, when the sound amplitude is sufficiently large, the bubble oscillates in a nonlinear fashion, causing volume change of 1 million times over each cycle, including a dramatic collapse [60,61]. The collapse of a tiny micro-meter size bubble can emit light – well known as sonoluminescence [60,61]. On the other hand, when the sound amplitude is small, the bubble oscillates linearly and stably over cycles. In this regime, instead of bubble collapse and violent flow, these vibrating microbubbles establish steady flow currents around them [33,62]. This type of steady flow is the result of the Reynolds stresses within a thin boundary layer, known as cavitation streaming or bubble microstreaming, a counterpart of the steady streaming induced over solid boundaries [63].

Steady streaming flow is a second-order effect, originating from the non-linearity of the
Navier-Stokes equations. To better illustrate the driving mechanism of this type of boundary induced steady streaming flow, in a two dimensional (2D) polar coordinate system, for an incompressible Newtonian fluid, we can write the Navier-Stokes equation in the form of streamfunction [5],

\[
\frac{\partial \nabla^2 \psi}{\partial t} - \frac{1}{r} \frac{\partial \left( \psi, \nabla^2 \psi \right)}{\partial (r, \theta)} = \nu \nabla^4 \psi, \tag{1.1}
\]

where \( \psi, \nu = \mu/\rho \) are the stream function and kinematic viscosity of the liquid,

\[
u_r = \frac{1}{r} \frac{\partial \psi}{\partial \theta}, \quad \text{and} \quad \nu_\theta = -\frac{\partial \psi}{\partial r},
\]

and

\[
\frac{\partial \left( \psi, \nabla^2 \psi \right)}{\partial (r, \theta)} = \frac{\partial \psi}{\partial r} \frac{\partial \nabla^2 \psi}{\partial \theta} - \frac{\partial \psi}{\partial \theta} \frac{\partial \nabla^2 \psi}{\partial r}.
\]

Introducing bubble radius \( a \), the inverse of angular driving frequency \( \omega^{-1} = 1/(2\pi f) \), and typical bubble interface velocity \( U \) as characteristic length, time, and velocity scales, Eq. (1.1) is non-dimensionlized as,

\[
\frac{\partial \nabla^2 \psi}{\partial t} - \frac{\epsilon}{r} \frac{\partial \left( \psi, \nabla^2 \psi \right)}{\partial (r, \theta)} = \frac{\delta^2}{2} \nabla^4 \psi, \tag{1.2}
\]

where

\[
\epsilon = \frac{U}{\omega a} \quad \text{and} \quad \delta = \sqrt{\frac{2\nu/\omega}{a}}.
\]

Here, \( \epsilon \) represents the dimensionless oscillation amplitude of the bubble interface with respect to the bubble size, radius \( a \); while \( \delta \) is the ratio of stokes boundary layer thickness to the bubble size.

For \( \epsilon \ll 1 \), the asymptotic solution of \( \psi \) in \( \epsilon \) is written as

\[
\psi(r, \theta, \delta, t) = \epsilon \psi_0(r, \theta, \delta, t) + \epsilon^2 \psi_1(r, \theta, \delta, t) + O(\epsilon^3). \tag{1.3}
\]
Substituting Eq. (1.3) into Eq. (1.2) and collecting terms of the same order, we obtain

\[ O(\epsilon) : \frac{\partial \nabla^2 \psi_0}{\partial t} = \frac{\delta^2}{2} \nabla^4 \psi_0, \]  

(1.4)

and

\[ O(\epsilon^2) : \frac{\partial \nabla^2 \psi_1}{\partial t} - \frac{1}{r} \frac{\partial (\psi_0, \nabla^2 \psi_0)}{\partial (r, \theta)} = \frac{\delta^2}{2} \nabla^4 \psi_1 \]  

(1.5)

Taking a time average of Eq. (1.5) over one period cycle, \( T \), i.e. \( \langle \cdot \rangle = \frac{1}{T} \int_0^T (\cdot) dt \), we have

\[ \langle \nabla^4 \psi_1 \rangle = \nabla^4 \psi_s = -\frac{2}{\delta^2} \left\langle \frac{1}{r} \frac{\partial (\psi_0, \nabla^2 \psi_0)}{\partial (r, \theta)} \right\rangle. \]  

(1.6)

The right-hand-side term in Eq. (1.6) is non-zero in the region of non-zero fluctuating vorticity. And this driving force can therefore be interpreted as a second-order effect (\( \sim \epsilon^2 \)) in the amplitude of first-order (\( \sim \epsilon \)) oscillatory flows. The streaming Reynolds number is an important parameter characterizing this secondary steady flow, defined as \( Re_s \equiv \frac{2\pi \epsilon^2 a^2 f}{\nu} \), where \( f \) is the driving frequency [63, 64]. In visualizing the flow fields with various particle tracking methods, we are primarily concerned with \( \psi_1 \), the steady component of the flows, which is also most relevant to microfluidic applications. Therefore, in most cases, we only need to choose a sampling rate much slower than the driving frequency of the acoustic pressure. In cases to understand the fast time-scale bubble dynamics, we are also able to gather sufficiently accurate temporal and spatial resolutions with our high-speed camera (up to 100,000 frame per second).

Although earlier work of microbubble streaming flows dates back to the studies by Elder in the 1950s [62] and some work by Rooney in the 1970s [33], it is only during the last decade that microbubble streaming flows have revived and emerged as an increasingly popular actuating mechanism for microfluidics, much due to the work by Hilgenfeldt and his group. Following Longuet-Higgins’ approach [65], Marmottant and Hilgenfeldt provided an analytical approach to describing the streaming flow arising from a three dimensional (3D)
hemispherical oscillating microbubble absorbed or attached on a solid wall [35]. In their approach, the presence of the solid wall is represented by adding additional Stokes singularities to satisfy the vanishing velocity boundary condition on the solid wall. In this scenario, the far-field flow is dominated by a dipole-like term. Subsequently in their several other theoretical, experimental and numerical studies, they measured the phase shift of an oscillating bubble with ultra-fast speed imaging [66], and further demonstrated practical applications such as deforming/lysing vesicles [35], and directional transport of liquid [36–38].

A few other research groups have used microbubble streaming flows for microfluidic applications as well, with examples including mixing [39, 40, 67–69], liquid transport [70], cell/particle sorting [71], and particle trapping [72–74]. Liu et al. designed a reaction chamber with an array of 3D air pockets that utilizes microbubble streaming to enhance mixing, and subsequently applied the technique for DNA hybridization, demonstrating enhancement of both signal intensity and uniformity [67–69]. In addition to 3D bubbles, several other versions of mixing enhancement with 2D microbubbles were implemented by other groups as well, by either placing multiple side bubbles along a straight channel [40] or a single bubble inside a channel [39]. Additionally, Patel et al. demonstrated deflection of cells/particles trajectories at a Y-junction inside a microchannel by instantaneously activating the bubble streaming flows [41]. By breaking symmetry of the streaming flow, Tovar et al. placed angled later blind channels (or cavities) along the main channel and excited the liquid/air meniscus to induce steady streaming and directional transport of liquid [70, 71].

1.4 Organization of the dissertation

In this dissertation, we focus on the approximately 2D streaming flows arising from acoustically driven cylindrical microbubbles, a practically relevant configuration that is commonly encountered in LOC systems due to the lithography-based micro-fabrication technique. The dissertation research aims at both a quantitative understanding of 2D microbubble stream-
ing flows and exploration of these flows as a versatile microfluidic toolbox for microparticle manipulation and liquid mixing. In Chapter 2, we will begin by describing the experimental set-ups, design and fabrication of bubble-based microfluidic devices as well as the image and data analysis techniques. In Chapter 3, we will describe and explain how to use microbubble streaming flows to shape the flow topology and thus selectively trap micro-particle by size. Exploiting the trap-and-release mechanism, we will further demonstrate practical applications: switching, sorting, enriching/pre-concentrating, and focusing. In Chapter 4, we will experimentally characterize the oscillation modes and the frequency response spectrum of acoustically driven bubbles and establish a correlation between the frequency dependent bubble dynamics and the resulting streaming flow patterns. In Chapter 5, we will show that the fundamental understanding can guide better designs for applications: various strategies for efficient bubble-based micro-mixers. In Chapter 6, we will conclude the major findings from this dissertation research and present perspectives of ongoing and future work.

In the Appendix, we present a brief summary of ongoing collaborative work with Professor Jimmy Hsia’s group on the study of liquid droplets on micro-patterned surfaces. This part of work is not directly connected to the main theme of microbubble streaming flows, but it does share the broader topics of solid-liquid interaction, interfacial flows, and dynamics of contact lines. While the ideas discussed in the Appendix are primarily from Professor Hsia and his graduate student Huan Li, the experimental implementation, data acquisition, and processing have been a collaborative effort of both groups.

1.5 Key accomplishments

This dissertation work presents a systematic study on microbubble streaming flows – covering both advances in practical microfluidic device designs, fabrications and applications, and progress in the fundamental understanding of the fluid physics. Key accomplishments are summarized as follows:
• A novel concept of flow control with microbubble streaming flows. Without introducing mechanical and moving parts, acoustically driven microbubbles are able to generate strong local flows to divide liquid flows into extremely different domains – regions of closed streamlines and region of open streamlines. This unique flow partitioning creates a virtual gap between bubble interface the upstream vortex.

• Applying such tunable flows as adjustable tools for microparticle manipulations. The thin gap structures are capable of selectively trapping micro-sized objects by size and lead to versatile applications – filtering, focusing, switching, and sorting. Furthermore, this type of control is flexible and tunable through adjusting driving amplitude and frequency. This general concept is non-invasive, simple and passive, and has potential applications to separation, purification, sorting of biological objects, including cells, vesicles and DNA.

• Understanding bubble dynamics and streaming flow patterns from first principle fluid dynamics. High-speed imaging analysis of fast-time first order bubble motion has revealed the link between the shape modes and steady flow patterns. In a low frequency regime, mixed mode streaming resulted from interaction of oscillating flows of two shape modes is dominant – causing “fountain” flows; while at high frequency, wall streaming resulting from oscillating flows over solid walls is the main mechanism of the “anti-fountain” far-field flows. Measurement of bubble dynamics and characterization of streaming flow with varying geometric parameters of the bubble configuration provide valuable information and guidelines for predicting resonance frequency, flow speed, and flow topology. The findings not only have explained the rich and complex flow patterns arising from oscillating bubbles, but also have provided researchers of other fields, particularly experimentalists, with guidelines for designing proper flows to suit specific applications.

• Innovative design of more efficient bubble-based micro-mixers. A thorough under-
standing of the physical principles has enabled general improvement of mixers based on microbubble streaming flows. Various microfluidic mixers are fabricated and mixing characteristics are measured. The proposed general classes of solutions – modulating acoustical driving patterns and proper arrangement of multiple bubble elements have both improved the mixing performance compared to the existing ways of using microbubble streaming flows. The two strategies demonstrate the flexibility of using microbubble streaming flows as both active and passive micro-mixers.
Chapter 2
Experiment

In this chapter, we describe the details of the experimental set-ups, which include design and fabrication of microfluidic devices, equipment, materials, visualization methods as well as data analysis.

2.1 General fabrication techniques of microfluidic devices

Fabrication of microscale fluidic devices represents an essential and enabling part of microfluidics. The fabrication techniques have gone through revolutions since the inception of microfluidics. Until the middle 1990s, silicon/glass based micro machining techniques have been the primary means to make these micro/nanoscale geometries [75–79]. This is mainly because microfluidics was originally derived as a subfield of MEMS and the microelectronics fabrication methods are well established in the semiconductor industry [1]. With the rapid development of microfluidics, and in particular the interaction of microfluidics with other fields such chemistry and biology, traditional silicon/glass based micro machining have experienced several limitations: relatively high cost, lack of optical opacity, and requirement of highly specialized skills and expensive equipment.

There is thus an urgent demand for inexpensive and disposable materials, and simple techniques for making microfluidic devices. Instead of using silicon/glass, making devices from plastic or polymeric materials see many benefits: reduced cost, simpler manufacture, and a
wide range of materials available as well as fast turn around time for making prototypes [80]. Researchers have developed various alternative micro-fabrication technologies to build microfluidic devices, such as laser ablation of polymers [81], polymeric laminate [82], injection molding [83], and molding with elastomeric materials [84]. Among these, soft-lithography with Polydimethylsiloxane (PDMS) pioneered by Whitesides’ group at the Harvard University is one of the most commonly used techniques, especially suitable for academic research settings, allowing for inexpensive and quick fabrication of prototype devices [85–87].

2.2 Fabrication with soft-lithography

Broadly speaking, soft-lithography refers to a family of techniques for micro-fabrication based on printing and molding using elastomeric stamps with the patterns of interest. And there is plenty of information and vast literatures on soft-lithography [85–87]. Here we will describe the specific details and parameters that are important and used in this dissertation research, which will serve as a reference for future use.

2.2.1 Basic process flow of soft-lithography

The basic process flow of soft-lithography technique consists of: (i) design and fabrication of photomask, (ii) patterning of photo-resist (SU-8) on silicon wafers, (iii) replication of PDMS from the SU-8 mold (or template), (iv) sealing of PDMS replica to form closed microfluidic channels. The general process flow is schematically shown in Fig. 2.1. For the processes in step (ii), we use the cleanroom facilities (hotplate, spin-coater, mask aligner and molecular vapor deposition system) in the Micro-Nano-Mechanical Systems Cleanroom Laboratory (MNMS) within the Department of Mechanical Science and Engineering at the University of Illinois.
2.2.2 Photomask

We design 5" by 5" chrome photomasks with software AutoCAD, and subsequently send the AutoCAD design files to FineLine Imaging (Colorado Springs, CO) for plotting, typically with a 10 μm resolution. For processes that require two or more layers of structures, these photomasks need to be aligned, so that we also need to draw alignment marks on all photomasks. In our initial phase of research, we also explored the possibility of using high-resolution mask printed on plastic transparency. Although the cost of transparency mask is much cheaper (∼ 20 times) than the chrome mask, the result was not satisfactory because of the very rough surfaces. When used for microbubble streaming experiments, the
rough surfaces of solid boundary walls cause local steady streaming flows near them.

2.2.3 SU-8 mold

In general, a master mold (or template) for PDMS replication can be made either into silicon wafer through deep reactive ion etching, or by forming desired photoresist structure directly on silicon substrate. The former method is often more expensive and takes a longer time. Therefore the latter method is preferred and used in this work. A number of photoresists such as the AZ series products from Microchemicals and the SU-8 photoresist from Microchem are good candidates for this purpose. Here we choose SU-8, a negative epoxy based photoresist. SU-8 have good mechanical properties, including high melting temperature and good mechanical strength. Moreover, SU-8 photoresist is available in different viscosities, so that a wide range of thickness (from a few $\mu$m to several hundred $\mu$m) is possible with a single spin-coating step.

Starting from a 4" bare silicon wafer, we first clean the wafer with acetone followed by Isopropanol (IPA), and then blow dry the wafer with compressed Nitrogen gas. Clean and dry silicon wafer is a key to achieve uniform photoresist thickness with spin-coating and to ensure good adhesion to silicon wafer in the photoresist development process. To ensure the silicon wafer is completely free of moisture, we do a dehydration bake by placing the wafer on a hotplate at 150-200$^\circ$C for 10 minutes.

Upon removal of the wafer from the hotplate, we allow the wafer to return to room temperature (about 5 minutes). Photoresist SU-8 is spin-coated onto the silicon wafer to 100 $\mu$m thickness. Depending on the type of SU-8, this thickness can be achieved by spinning at 3000 rpm with SU-8 2100, 2125 rpm with SU-8 2075, or 1600 rpm with SU-8 2050 (refer to SU-8 product sheets for details).

We want to make a few important notes about processes dealing with thicker ($\geq 100 \mu m$) SU-8, which are based on our own experiences as well as previous literature [88, 89]. To ease the pouring process of the thick and viscous SU-8 (e.g. SU-8 2100 or 2075) from the
bottle, we first heat the SU-8 bottle to 50°C for 30 min to lower the viscosity (and also to remove air bubbles if there are any). After pouring the heated SU-8 onto the center of silicon wafer, it is crucial to wait for enough time (at least 5 minutes) to cool SU-8 down to room temperature and to recover its room temperature viscosity, which will then give the right spin-coating thickness.

According to the standard SU-8 data sheet, soft baking first at 65°C and immediately followed by baking at 95°C for specific time will make SU-8 ready for exposure. However, in practice, we find it more advisable to do the soft baking in different procedures to achieve a uniformly smooth surface with few bubbles. Here, we place the wafer spin-coated with liquid SU-8 on a leveled hotplate at 50°C for half an hour and cover it with a glass petri dish. The petri dish cover creates an environment with saturated solvent that can prevent cracking on the outer surface of SU-8. Additionally, at 50°C the viscosity is significantly lowered so that SU-8 reflows and achieves a uniform layer across the whole wafer. Note that it is important to place it on a perfectly leveled hot plate.

While keeping SU-8/wafer covered with a petri dish, we next soft bake the wafer at 65°C for 5-10 minutes, and slowly ramp up the temperature (at a rate of 0.5-1°C/min) to 95°C and hold at 95°C for 30 mins. Additional baking at 95°C for 30 mins is performed without the petri dish. We keep the wafer on the hotplate and let it cool down naturally to room temperature by turning off the hotplate. This will avoid thermal shock and minimize the stress and possible cracks.

The remaining steps – exposure, post baking, development, and hard baking – will follow the standard procedures provided by the SU-8 data sheet. Note that when using Mask Aligner to expose the SU-8, we choose 2× as the relative exposure dose. When develing the mold in SU-8 developer solution, spaying IPA onto the wafer is a convenient way to check whether the development is fully completed. If there is undeveloped SU-8 residue, IPA will cause the residue to turn white. After development, a hard baking at 200°C is done for 30 mins. Hard baking will remove any small cracks at the sharp corners of the mold. Finally, to
avoid adhesion to the Silicon/SU-8 mold and to facilitate the peeling of PDMS replica from SU-8 mold, we also apply a layer of perfluorodecyltrichlorosilane (FDTS) with a Molecular Vapor Deposition System.

The above steps are for a single layer SU-8 mold. When creating top microbubbles, which are not within the same layer as the main channel, we then need to fabricate two-layer SU-8 molds. After the first layer is exposed, a second layer process is repeated. The processes are similar, except that the first layer of exposed SU-8 is not developed until the second layer is exposed and post baked. The final development step is then done for both layers simultaneously [89].

### 2.2.4 PDMS replication

Having obtained the SU-8 master mold, the rest of the fabrication processes can be carried out outside the cleanroom. The silicon/SU-8 mold is placed inside a petri dish or a self-made aluminum bowl on a leveled surface (here on an optical table). Two components of PDMS (Sylgard 184, Dow Corning) are well mixed at ratio 10:1, degassed with a vacuum chamber, and poured onto the SU-8 mold. PDMS cross links in different times at different temperatures, ranging from a few hours to a day. We often use room temperature because of the low shrinkage. At the room temperature, it takes 24 hours for PDMS to cure.

Fully cured PDMS replicas are peeled off from the SU-8/silicon wafer and cut into individual pieces. Inlets and outlets are punched using a 1.5 mm Biopsy punch (Premier Uni-Punch). Then we bond the PDMS replica to a flat PDMS layer (casted from a bare silicon wafer) with the help of surface treatment by a corona discharger (Laboratory Corona Treater, Chicago, IL). This hand-held corona discharger is a convenient and cost-effective (\(\sim\) $500) replacement to a conventional plasma cleaner (\(\sim\) $10000). In fact, it also has certain advantage over the more sophisticated oxygen plasma. Unlike the immediate permanent bonding with oxygen plasma, the surface bonding treated by the corona discharger is reversible even after a few minutes of initial contact, and thus allows to remove and re-align...
if necessary.

While it is necessary to activate PDMS surface to enable permanent bonding between
PDMS and PDMS/glass, over treatment of plasma will often result hardening of the PDMS
surface and poor bonding. In our experiment, we find that 30s to 45s exposure achieves the
best bonding strength. It is important to bring the two treated surfaces into contact imme-
diately after surface treatment, because the surfaces will revert to their original untreated
state after 10 minutes or so. Immediate baking at higher temperature (50 to 60°C) in an
oven further increases the bonding strength. The PDMS microfluidic device is then bonded
to a substrate slide (either glass or polystyrene) after the same treatment with the corona
discharger. The inlets and outlets are interfaced through 1/32” inner diameter (ID) tubing
(SmallParts Inc) for liquid access.

2.2.5 Assembly of microfluidic devices

We adopt a modular design by building microfluidic device onto one substrate slide (i.e.
top substrate) and attaching a piezoelectric transducer (Physik Instrumente, Germany) to
another substrate slide (i.e. bottom substrate), in Fig. 2.2(a). These two substrate are
conveniently aligned and secured with common paper clips (Fig. 2.2(b)). Such a design
allows for easy and flexible mounting of different fluidic device onto a single bottom substrate,
eliminating the need for gluing a piezoelectric transducer onto every fluidic device substrate.
One typical snapshot of the fabricated device is shown in Fig. 2.2.

2.2.6 Bubble size control

As we will see in the later chapters, bubble size is a key factor pertaining to the bubble
dynamics as well as the resulting streaming flows. When exposed to typical microscope
illumination light, the liquid inside the microchannels will experience temperature rise. As-
suming the liquid is saturated with air, temperature rise will cause less solubility of air in
liquid, and consequently lead to growing bubbles. Previously, Marmottant et al. has shown that the bubble size can be controlled by adjusting the gas concentration in the liquid. By mixing water of different temperatures to have a slight supersaturation, they have maintained a stable and almost hemispherical bubble for hours [35–37]. In our experimental set-up here, the liquid in micro geometries is more sensitive to heating from the microscope illumination. Under normal imaging conditions, we would observe appreciable bubble size growth, as shown in Fig. 2.3(a). Inspired by the technique of Marmottant et al. [35–37], we build a chamber outside the PDMS microfluidic device, in Fig. 2.2. When filled with several ml of water, this water reservoir can slow down the temperature rise and thus stabilize the bubble size over a longer period of time (Fig. 2.3). Furthermore, by filling water of lower temperature, we can even decrease the bubble size. By manually adjusting both the volume and temperature of water in this chamber, we are able to maintain a relatively good control of the bubble size over a much longer period time, a time scale larger than the experimental time scale. We are also developing a more sophisticated chamber attachment with integrated heating element and a cooling function. Such an attachment can be easily mounted with our existing microfluidic device and provides a better and convenient control of water temperature in the chamber as well as the bubble size.
Figure 2.3: Snapshot of a microbubble at several time series under the illumination of the microscope light. (a1)-(a3) show the growth of the bubble when the PDMS chamber is empty. (b1)-(b3) show well-controlled bubble size (with a slightly visible decrease) by filling water into the chamber.

2.3 Equipment and materials

The schematic of the complete experimental setup is shown in Fig. 2.4. We mount a microfluidic device securely onto a movable microscope stage, which is controlled through the joystick or the Labview control program through the computer. A syringe pump (PHD Ultra, Harvard Apparatus) is used to infuse liquid solution into the fluidic channel through inlets at constant flow rate. The piezoelectric transducer (thickness 1 mm, diameter 10 mm, Physik Instrumente, Germany) provides acoustic driving to the system, using sinusoidal signals of frequency $f = 1 - 100$ kHz from a function generator (7075, Hioki, Japan) and an amplifier (7500, Krohn-Hite, USA). The device is illuminated by a halogen source (TH4-100, Olympus, USA) for transmitted-light bright-field microscopy. Additionally, a mercury vapor lamp is also available for fluorescent imaging. We use a high-speed camera (Phantom v310, Vision Research, USA) to capture top-view images or videos through an inverted microscope (IX71, Olympus) with a choice of 4×, 10×, 20× or 40× microscope objective lens.
Figure 2.4: Schematic of the experimental set-up. The microfluidic device is mounted securely onto a movable microscope stage. The syringe pump is used for infusing liquid into the fluidic channel. The piezoelectric transducer using signals from the function generator and amplifier induces driving pressure to the bubble. The high-speed camera captures videos or images through an inverted microscope objective lens. Images and videos are transferred to the computer for analysis.
2.4 Visualization and data analysis

2.4.1 Particle tracking and streak visualization

To visualize the flow field, we seed a density-matched water-glycerol solution (23% glycerol w/w) with polystyrene microparticles \( \rho_p = 1050 \text{ kg/m}^3 \), Magsphere Inc) as tracers. Different sizes are used, with the most common being particle radii of \( a_p = 1, 2.5, \) and \( 5 \mu \text{m} \). The polydispersity of each kind of particle is \(< 5\% \) (coefficient of variation of the radii). The surfactant Tween 20 (Fisher Scientific) is added at 1\% w/w to the liquid solution to prevent sticking of particles to the microchannel walls, as well as to avoid particle aggregation (the concentration of surfactant is well above the critical micelle concentration, so that surface concentrations should remain stable).

The high-speed camera is capable of recording at fast enough frame rate and with very short exposure time (minimum \( 1\mu \text{s} \)), and thus allows for visualizing both the oscillating bubble and the steady streaming flow. After saving the video files to computer, we use the programs ImageJ [90] and MATLAB for data analysis [91]. Streak photographs are obtained by superposing a series of typically 1000 successive images that are captured at a frame rate of 100 to 1000 frames per second (fps). To track particle trajectories, we use ImageJ [92] software for particle detection. First, the recorded images are converted to binary images by proper thresholding. Then, the built-in function “Analyze Particles” is used to detect the outline and to calculate the center of mass of the particles. The program works well to track isolated particles. When particles touch each other or the boundaries, the program fails to identify the correct size and therefore the particles. In our experiments, the particle concentrations are low enough and the video frame rates are high enough to ensure that trajectory tracking works well. The extracted position information is used to determine the velocity. In Fig. 2.5, we show a typical example of particle streak image, particle tracking, and the total velocity of the particle as a function of distance from the bubble center.
2.4.2 Particle imaging velocimetry

Particle imaging velocimetry (PIV) is a non-intrusive flow visualization technique to acquire velocity fields by seeding the fluid with tracer particles [93–95]. Assuming that these small tracer particles follow the flow faithfully, consecutive images with certain time delay can be used to determine the displacement as well as the velocity. With the high-speed capability of our camera system, and by seeding tracer particles at a higher concentration, we could perform PIV measurement to obtain the full field velocity and vorticity. We have successfully calibrated the PIV technique by measuring velocity at the middle plane of a rectangular microchannel, see Fig. 2.6(a). Fig. 2.6(b) shows the velocity and vorticity contour around an oscillating bubble driven at $f = 14.4$ kHz.

2.4.3 Astigmatism particle tracking velocimetry

Conventional PIV is capable of measuring a two dimensional velocity field, which is often the character of streaming flows from our experimental set-ups. However for different applications, e.g. mixing, 3D flows are more advantageous. As will be shown in Chapter 5, we can also easily construct 3D flows, by placing neighboring microbubbles into different planes ($x$-$z$ and $x$-$y$), and through the interaction of these streaming flows. To understand
and characterize the 3D flow structure as well as the implication on mixing, it is thus necessary to access the 3D velocity field. In collaboration with the group of Christian Kähler at Universität der Bundeswehr Munich (Germany), we employ astigmatism particle tracking velocimetry (APTV) to obtain 3D positions of tracer particles. APTV uses a cylindrical lens that distorts the images of tracer particles in a systematic way depending on depth (distance along the optical axis) [96–98]. The three dimensional particle trajectories can be quantitatively tracked and extracted. Fig. 2.7 shows a preliminary result of our collaborative work that demonstrates the feasibility of this method to measure bubble microstreaming flows [99]. In addition to characterizing mixing, the APTV method is also useful to assess the 2-D character of the flows (how large are 3D artifacts?), and refer to sec. 4.3.2 for further details on this question.

### 2.4.4 Imaging bubble motion

To study the dynamics of the bubble interface, we capture images of 120 × 80 pixels, at 100,000 fps and with an exposure time of 1 µs. Even though the camera sampling rate is almost the same as the higher driving frequencies $f \sim 100$ kHz, the very short exposure time
(a) volumetric particle trajectories in microbubble streaming flows, and (b) a projected view of particle trajectories in the $x$-$z$ plane.

(1 $\mu$s) and carefully chosen driving frequencies allow us to improve the time resolution using stroboscopic technique. We illustrate how this technique works with an example, in Fig. 2.8. When the bubble is driven at $f = 23.1$ kHz, with a sampling rate of 100,000 fps, the camera is able to capture four snapshots within one driving period $T_f$, (Fig. 2.8(a)-(d)). Note that
Figure 2.8: Improving time resolution with stroboscopic technique: (a)-(d) four consecutive snapshots captured at 100,000 fps of an oscillating bubble driven at $f = 23.1\, \text{kHz}$; (e) radius change at $45^\circ$ with respect to the initially undisturbed bubble; (f) improved temporal resolution by restacking the data points into one period $T = 1/f$.

the sample rate here cannot be divided by frequency $f$. Assuming the bubble is oscillating periodically, we can treat the bubble shape at a later time $t = nT + t_1$ ($n$ is an integer) the same as that of time $t = t_1$ and $t_1 < T$. Thus, by restacking the images from time $t > T$ back to a time duration of one period $T$, we are able to improve the time (or temporal) resolution. Fig. 2.8(e) and (f) show the radius change with respect to the initially undisturbed bubble ($\Delta r = r(t) - r_0$) at $45^\circ$ from the first 50 captured images and the improved time resolution by restacking 500 images into one period. Fig. 2.8(f) also indicates that the bubble oscillating frequency is the same as the driving frequency $f$, without significant contributions from subharmonic or superharmonic frequency modes.

2.4.5 Characterization of liquid mixing

In addition to the bright transmitted light, the microscope is also equipped with a mercury vapor lamp (X-Cite 120) and an epi-fluorescence attachment for fluorescence imaging. This
is useful for characterizing mixing of microbubble based mixers. In the mixing experiments, two liquid streams are infused through the two inlets into a main channel. One liquid stream contains fluorescent particles or fluorescein molecules and the other liquid stream does not. Upon illumination by the mercury vapor lamp through an emission filter (460 nm), the fluorescent particle or fluorescein emits lights of a longer wavelength $\approx 520 \text{ nm}$, which are then captured by the high-speed camera. Under low concentration condition, the gray scale intensity (fluorescent signal $C$) of the image is proportional to the fluorescent particle or fluorescein molecule concentration. Thus the mixing quality between the two liquid streams can be quantified through the analysis of the gray scale intensity distribution of the captured images.
Chapter 3
Manipulation of microparticles

In this chapter\(^1\), we will explore and demonstrate a general concept of flow manipulation in microfluidic environments, based on controlling the shape and position of flow domains. Using microbubble steady streaming, we show that regulation of the relative strength of streaming flow and a superimposed Poiseuille flow allows for size-selective trapping and releasing of particles, with particle size sensitivity much greater than what is imposed by the length scales of micro-fabrication. A simple criterion allows for quantitative tuning of microfluidic devices for manipulation of particles of desired size. We further show how to design bubble microfluidic devices that use these concepts to switch, sort, filter, enrich, and pre-concentrate particles of selected sizes, either by concentrating them in discrete clusters (localized both stream- and spanwise), or by forcing them into narrow, continuous trajectory bundles of strong spanwise localization.

3.1 Introduction

Trapping, sorting and focusing of micron-sized objects such as biological cells, droplets and particles in microfluidic environments is an important preprocessing step in \(\mu\)TAS [8, 21] for single cell detection and diagnostic analysis. There are two main strategies of manipulating of micron objects: active and passive method. The former relies on actively applying various external force fields, including hydrodynamic (inertial) [101–103], electrokinetic [104–107], dielectrophoretic [108–111], magnetic [112, 113], optical [114, 115] or

\(^1\)This chapter is adapted from [42, 43, 100].
acoustic [116–118] forces. These forces will usually act differently on particles depending on their size, geometry, and mechanical or electromagnetic properties; as a result, particle separation and sorting becomes possible. A passive alternative to active methods is achieved through the integration of geometric features such as obstacles and side channels into the microfluidic network [119–121]. Direct contact and interaction between the objects and geometric features make size-dependent, directional and selective transports of particles and cells possible [122, 123]. In this scenario, the introduction of very small structural elements on the order of the particle/object size [119–123] are often necessary.

Instead of displacing surrounding liquid directly using volumetric change of the microbubbles, acoustically driven microbubbles rectify rapid oscillatory motion of the air/liquid interface into steady streaming flows in the bulk liquid around the bubbles. Such steady streaming flows are useful in deforming and lysing vesicles [35], directional transport of liquid [36, 37] and enhancing mixing in microfluidics [67, 124]. Bubble streaming flow takes a unique position in that the bubble interface is doubtless an active element, but its amplitude is usually very small compared to the flow dimensions. For the purposes of flow description the bubble is merely a passive boundary, however it adds an important component to the microfluidic flow by shaping the flow domain inside the micro devices.

Here, we integrate single or multiple microbubbles into microfluidic devices. Through bubble steady streaming, these bubbles shape the flow domain and allow us to manipulate particles by size. More specifically, bubble-induced microstreaming is used to trap particles in an H-shaped device for switching and sorting of microparticles (Setup B in Fig. 3.1). Moreover, we show that multi-bubble systems can be used for focusing in a continuous fashion (Setup C in Fig. 3.1). In addition, we discuss how these devices can be used to trap particles continuously for applications such as pre-concentration of particles, filtering and particle enrichment. The technique presented here is novel and has a number of advantages over the existing methods, including very simple manufacturing, interactive control, general applicability, and negligible heat generation.
3.2 Microfluidic device designs

We fabricate microfluidic devices in Polydimethylsiloxane (PDMS) using soft lithography [85], (refer to Chapter 2 for details). The devices connect one or more inlets and outlets to a main channel with a depth of \( D = 100 \mu m \) and height in the image plane (\( x-z \) plane in Fig. 3.1) of \( H = 250 \mu m \). The essential elements of the devices are one or more blind side channels, which are positioned perpendicular to the main channel. When aqueous solution is introduced into the main channel, each side channel reliably retains a gas pocket, which constitutes a semi-cylindrical air bubble [39] protruding into the main channel. The typical dimensions of these devices are \( w = 80 \mu m \) wide and therefore the resulting bubble radius \( a \approx w/2 = 40 \mu m \) (Fig. 3.1). Several typical designs are schematically shown in Fig. 3.1. Setup A consists of a straight main channel with one inlet (I) and one outlet (O) and a single side bubble located middle way; Setup B is an H-shaped device with two inlets (I₁, I₂) and two outlets (O₁, O₂), and with a side bubble located upstream the junction of outlets; Setup C consists a straight main channel with side bubbles placed alternatingly on both sides of the main channel.

3.3 Microbubble streaming flows: experimental observation and singularity modeling

Under the periodic pressure field induced by the piezoelectric transducer, the bubble oscillates at the driving frequency \( f \) and with an oscillation amplitude \( \epsilon a \), where \( \epsilon \) is the dimensionless oscillatory amplitude, typically in a combination of a dominant volume mode and surface modes [125]. The fast oscillatory motion of the bubble surface produces a second order streaming flow with a steady component, which is the time averaged motion over cycles. The steady streaming around the bubble typically has two symmetric closed-loop vortices above the bubble, in Fig. 3.2(a), similar to the flows generated from hemispherical
The maximum streaming flow velocity, $u_s$, is observed near the bubble surface, and increases quadratically with the increasing oscillation amplitude (i.e. driving voltage), as shown in Fig. 3.2(b). This conforms to the theoretical expectation that, as a second-order effect in $\epsilon$, the scale of streaming should be $u_s \sim \epsilon^2 a \omega$, where $\omega = 2\pi f$. The least square fitted prefactor of the quadratic law shows that dimensional analysis also predicts the prefactor of $u_s$ to be within 15% of the measured value (Fig. 3.2(b)).

The streaming flow is characterized by the streaming Reynolds number $Re_s \equiv u_s a / \nu$, where $\nu$ is the kinematic viscosity ($\nu \approx 1.8 \times 10^{-6}$ Pa s for our water/glycerol mixture) [126]. If $Re_s \ll 1$, the flow is known as Rayleigh-Nyborg-Westervelt (RNW) streaming [64], i.e., as a Stokes flow far from the bubble. It can be modeled using the method of images and singularity theory [35,127].

In the RNW streaming regime ($Re_s \ll 1$), the secondary steady streaming flows are
Figure 3.2: Streaming flow from a semi-cylindrical microbubble: (a) streak image \((f = 16.8 \text{ kHz})\) without superimposed Poiseuille flow; (b) streaming velocity scale \(u_s\) at different driving voltages. The prefactor (from least square fit) of 0.83 is close to the dimensional-analysis expectation of 1.

Figure 3.3: Polar coordinate system of the 2D geometry used in the calculation of microbubble streaming flow. The plane considered here is in \(x-z\), such that \(x = r \cos \theta\) and \(z = r \sin \theta\).

approximated by Stokes flows. As we will see in the next chapter, steady streaming flows arising from semi-cylindrical bubbles sandwiched between two plates are approximately two dimensional in the \(x-z\) plane. It is thus easier to work with stream function formulation approach by following methods in Ref. [35]. A polar coordinate system \((x = r \cos \theta\) and \(z = r \sin \theta)\) coaxial with the bubble is used, with the walls at \(\theta = 0\) and \(\theta = \pi\) (i.e. \(z = 0\)), schematically shown in Fig. 3.3.
For two dimensional Stokes flows of an incompressible, Newtonian fluid, the governing
equation is a Biharmonic equation in terms of stream function $\psi_s$ [100],

$$\nabla^4 \psi_s = 0 \quad (3.1)$$

and the velocity components are $u_r = \frac{1}{r} \frac{\partial \psi_s}{\partial \theta}$ and $u_\theta = -\frac{\partial \psi_s}{\partial r}$ respectively. For the bubble
configuration in our experimental set-up, it is approximately a semi-cylindrical bubble at-
tached to a solid wall (in Fig. 3.3). Thus, the no-slip boundary condition ($u_s = u_\theta = 0$) is
valid at this solid wall, i.e.,

$$\frac{1}{r} \frac{\partial \psi_s}{\partial \theta} = \frac{\partial \psi_s}{\partial r} = 0 : \quad \theta = 0, \pi. \quad (3.2)$$

By separation of variables, general solutions (singularity solutions) to Eq. (3.1) satisfying
the no-slip boundary condition in Eq. (3.2) are obtained as,

$$\psi_s(r, \theta, n) = \frac{1}{r^n} \left\{ \frac{1}{2} \left[ 1 + (-1)^{1+n} \right] \left[ \cos(n\theta) - \cos((2 + n)\theta) \right] \right\}$$

$$+ \frac{1}{2} \left[ 1 - (-1)^{1+n} \right] \left[ \sin(n\theta) - \frac{n}{2+n} \sin((2 + n)\theta) \right], \quad (3.3)$$

where $n = 1, 2, 3, \ldots$.

With Eq. (3.3), the first few solutions are written as follows,

$$\psi_s(r, \theta, 1) = \frac{1}{r} \left( \cos \theta - \cos(3\theta) \right) \quad (3.4)$$

$$\psi_s(r, \theta, 2) = \frac{1}{r^2} \left( \sin(2\theta) - \frac{1}{2} \sin(4\theta) \right) \quad (3.5)$$

$$\psi_s(r, \theta, 3) = \frac{1}{r^3} \left( \cos(3\theta) - \cos(5\theta) \right) \quad (3.6)$$

$$\psi_s(r, \theta, 4) = \frac{1}{r^4} \left( \sin(4\theta) - \frac{2}{3} \sin(4\theta) \right) \quad (3.7)$$

$$\psi_s(r, \theta, 5) = \frac{1}{r^5} \left( \cos(5\theta) - \cos(7\theta) \right) \quad (3.8)$$
The streamlines associated with these no-slip singularity solutions are shown in Fig. 3.4(a)-(e).

![Streamlines of the general solution](image)

Figure 3.4: Streamlines of the general solution: (a) \( \psi_s(r, \theta, 1) \), (b) \( \psi_s(r, \theta, 2) \), (c) \( \psi_s(r, \theta, 3) \), (d) \( \psi_s(r, \theta, 4) \), (e) \( \psi_s(r, \theta, 5) \). (f) with proper coefficients chosen, the truncated series solution (Eq. 3.9) that uses seven terms is able to account for the presence of the bubble.

The solution to Eq. (3.1) is a linear superposition of the singularity solutions \( \psi_s(r, \theta, n) \), i.e.

\[
\psi_s(r, \theta) = \sum_{n=1}^{\infty} C_n \psi_s(r, \theta, n).
\]  

(3.9)

To satisfy no-stress boundary condition on the bubble surface, we choose coefficients to
minimize the shear stress on the bubble surface. As $n$ increases, the velocities of higher order singularity solutions decays faster away from the bubble, thus only several singularity solutions are needed to describe the flow field accurately. With proper coefficients chosen, seven singularity solutions yield streamlines that represent the presence of the bubble, as shown in Fig. 3.4(f).

To account for the second wall that encloses the bubble, the method of images is used to ensure the no-slip boundary condition on the second wall, i.e. $z = H$. This method requires adding image singularities repeatedly [35]. The streamlines are calculated with the same geometry as in experiment, in Fig. 3.5. There exists qualitative agreement between singularity theory and experiment. The singularity theory described here is developed for a single bubble configuration. However, because of the low Reynolds number of our system, external Poiseuille flow can be superimposed (in Fig. 3.5(b)(d)), and multiple bubble systems can be built (in Fig. 3.5(c)). With the addition of a Poiseuille flow (from right to left), the symmetry of the flow field is broken (in Fig. 3.5(b)(d)), and the flow consists of regions of closed and open streamlines.

Note that in our experiment, the condition $Re_s \ll 1$ is only met for the smallest of our experimental frequencies and/or small driving voltages. For the most typical frequencies $f$ used in experiment, between 10 kHz and 30 kHz, $Re_s \sim 1$ for $\epsilon \approx 0.05$. Despite the implication $Re_s = 0$, RNW theory quite accurately describes qualitative aspects such as the streamline picture, see the comparison between the experiment streak image (Fig. 3.2(a)) and the theoretical calculation (Fig. 3.5). This qualitative agreement is not surprising, because it is well-known that Stokes flow theories tend to be pretty solid (even quantitatively) up to Reynolds number about 10 or so$^2$.

The singularity theory approach so far is heuristic in the fitting of coefficients in Eq. (3.9). In the chapter (sec 4.4), we will outline an asymptotic theory that accounts for the full Naiver-Stokes equations and derives streaming flows directly from the bubble motion [43, 128]. But

$^2$Personal communication with Stephen H. Davis.
Figure 3.5: Streamlines calculated from RNW singularity theory. The separation of the two walls is the same as that of experiment, $H = 6.25a$. (a) a single bubble between two walls; (b) with an imposed Poiseuille flow (right to left as indicated the arrow); (c) two bubbles attached on two separation walls; and (d) a two-bubble configuration with an imposed Poiseuille flow. The open streamlines are indicated by blue streamlines, and the closed streamlines are red.
Figure 3.6: Particle trajectories of large and small particles. (a) initial positions of two different-sized particles; (b),(c) resulting closed trajectories, showing that large particles stay closer to the bubble.

the present formalism gives a good qualitative idea and is correct in predicting the shape of the flow field for the frequency range between 10 kHz and 30 kHz. The singularity theory is especially useful for guiding device design by providing a quick way of visualizing the flow field.

3.4 Manipulating particles

3.4.1 Size dependent behavior in bubble streaming flow

In pure bubble streaming flows, finite size particles show size-dependent behavior around the bubble. As illustrated in Fig. 3.6, two particles of radii $a_p = 2.5 \, \mu m$ and $a_p = 5 \, \mu m$ are almost at the same initial position when the bubble is excited. As the particles are transported in the streaming flow, they settle onto different stable characteristic closed trajectories (loops) around the bubble. The large particle orbits on a smaller trajectory, while the small particle orbits on a larger loop.

Though small in size and density-matched, the microparticles are not completely passive tracers. In addition to the Stokes drag force, several other forces may be important, such as
Saffman lift and Faxén force [129–131]. Taking streaming velocity \( u_s \), bubble radius \( a \), and particle radius \( a_p \) as characteristic velocity scale, length scale and particle size respectively, we can estimate the magnitude of these forces: Stokes drag (\( F_{\text{drag}} \sim \pi \mu a_p u_s \)), Faxén force (\( F_f \sim \pi \mu a_p^3 u_s / a^2 \)), and Saffman lift (\( F_s \sim (\rho \mu)^{1/2} a_p^2 u_s^{3/2} / a^{1/2} \)). Even with the largest particle size \( a_p = 5 \mu m \), we find that both Saffman lift and Faxén force are much smaller than the Stokes drag (\( F_f, F_s < 0.01 F_{\text{drag}} \)). Therefore, the particles are able to follow the streamlines like passive tracers faithfully. However, near the bubble surface the streamlines are denser. The presence of the bubble restricts the particle’s ability to stay on the same streamline, because the particles cannot penetrate the bubble surface [42]. Consequently, particles are forced to move to another streamline due to the finite size effect of the particles.

### 3.4.2 Shaping flow domain and selective trapping of microparticle by size

In practical applications, microfluidics often require throughput through a device. In this subsection, we show how to use microbubble streaming together with a pressure gradient flow driven by a syringe pump (i.e. a Poiseuille flow in rectangular channels) to achieve such throughput and at the same time manipulate particles in these combined flows. The parameter used to regulate the flow is the relative streaming strength, \( s \equiv u_p / u_s \), defined as the ratio of the mean Poiseuille velocity \( u_p \) to the streaming velocity \( u_s \). Fig. 3.7 shows the evolution of the flow field as \( s \) varies. By this definition, a smaller \( s \) means stronger streaming flows compared to Poiseuille flow. In all images, the Poiseuille flow is directed from right to left (\(-x\) direction). When the bubble is not excited (\( s \to \infty \)), the flow assumes its Poiseuille characteristic with a parabolic velocity profile in the imaging plane. As the streaming velocity increases (i.e., \( s \) decreases), the streamlines bend towards the bubble upstream of the bubble and a closed loop region forms downstream of the bubble. With further increase of streaming velocity (smaller \( s \)), the streaming flow dominates near
Figure 3.7: Flow field of combined bubble streaming and main-channel Poiseuille flow (Poiseuille flow from right to left): (a) bubble is not excited; (b) $s = 0.17$; (c) $s = 0.043$; (d) $s = 0.021$; (e) $s = 0.014$; (f) $s = 0.009$. In (d), a critical streamline (red line) separates the flow domain into two parts.

the bubble with two vortex loop structures. At the upper edge of the upstream loop the flow has a hyperbolic point $P$ (Fig. 3.7(d)) with an associated critical streamline separating closed (vortex) and open (transport) streamlines. Far upstream, this critical streamline must be horizontal. The bubble streaming flow thus shapes the Poiseuille flow domain into two partitions divided by the critical streamline. In the flow region above this critical streamline, the Poiseuille flow is affected little with only slightly bent streamlines. In the flow region below this critical streamline, the open streamlines of the Poiseuille flow have to pass through the gap between the upstream closed loop and the bubble surface. Without introducing moving parts, the flow is thus divided into two domains, one of which experiences an extreme constriction, which is not imposed by a material boundary. By increasing the streaming velocity (smaller $s$), this constricted flow domain passing through the gap increases.

When particles are introduced into the combined flow, we observe selective trapping of
microparticles. In Fig. 3.8, at larger $s$, both the large and small particles pass by the bubble without being trapped; at smaller $s$, the large particle is trapped around the bubble. Significantly, while the smallest geometrically imposed length scale is $w = 80 \mu m$, the device efficiently separates mixtures of particles down to $1 \mu m$ and $2.5 \mu m$ radius, respectively. Note that both the absolute particle sizes and size differential are much smaller than the geometric length scales of the fluidic device ($w = 80 \mu m$, $H = 250 \mu m$), demonstrating a unique capability of handling micron-objects without relying on device of comparable size.

### 3.4.3 Mechanism of trapping

The trapping mechanism can be explained from a geometry argument [42]. Fig. 3.9(a) shows the streak image of the combined Poiseuille and bubble streaming flow fields. Far away from the bubble (at large $|x|$), the Poiseuille flow with mean velocity $\bar{u}_p$ dominates. Near the bubble, the streaming flow dominates with its two vortex loops, of which the upstream loop turns clockwise. At the upper edge of this upstream loop the flow has a hyperbolic point (Fig. 3.9(a)) with an associated critical streamline separating closed (vortex) and open (transport) streamlines. Far upstream, this critical streamline is horizontal at a height $z = h(s)$. Continuity requires that the streamlines below it (those at $0 \leq z < h$ for large $x$,
with a total flow rate of \( Q \) must pass inside of the closed loops, close to the bubble, while they cannot penetrate the bubble itself. Thus, all of \( Q \) must pass through a narrow gap of width \( d_{\text{gap}} \) between the bubble surface and the critical streamline (Fig. 3.9(b)). The smaller the parameter \( s \equiv \bar{u}_p/u_s \), the larger is \( h \), and the greater a fraction of the entire channel is funneled through the gap. Since the particle cannot penetrate the bubble, particles with radii \( a_p > d_{\text{gap}} \) will be pushed away from the bubble and into the vortex – these particles are then trapped.

![Image](image.png)

Figure 3.9: Mechanism of trapping: (a) streak image highlighting the hyperbolic point \( P \) and critical streamline (setup A, \( s \approx 0.04 \)); (b) schematic detail of the boxed region of (a).

The velocity within the narrow gap is close to uniform, \( u_{\text{gap}} \approx u_s \), so that \( Q = u_s d_{\text{gap}} \). Equating this to the Poiseuille flow rate to height \( h \) yields a theoretical prediction for the gap width, \( d_{\text{gap}} = sh\left[3(h/H) - 2(h/H)^2\right] \), which is the predicted critical particle radius for trapping to occur. Experimentally, by measuring \( h \) and \( u_s \) under different driving voltages, we find this prediction quantitatively confirmed. Table 3.1 and Fig. 3.10 demonstrate that experimental particle trajectories indeed close when the driving voltage exceeds that for which \( d_{\text{gap}} = a_p \) is predicted.

### 3.4.4 Releasing of trapped particles

As multiple large particles are trapped around the bubble, particle-particle interactions become important (Fig. 3.11(a)). We have not investigated their precise nature (though
Table 3.1: Predicted values of $d_{\text{gap}}$ (setup A, $f = 13.7$ kHz, $\overline{u}_p = 0.64$ mm/s) under different driving voltages $V$.

<table>
<thead>
<tr>
<th>$V$</th>
<th>$u_s$ (mm/s)</th>
<th>$s$</th>
<th>$h/H$</th>
<th>$d_{\text{gap}}$ ($\mu m$)</th>
<th>Predicted trajectory</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>18.3</td>
<td>0.035</td>
<td>0.71</td>
<td>7.0 ± 0.5</td>
<td>OPEN</td>
</tr>
<tr>
<td>50</td>
<td>26.7</td>
<td>0.024</td>
<td>0.82</td>
<td>5.6 ± 0.1</td>
<td>OPEN</td>
</tr>
<tr>
<td>60</td>
<td>34.8</td>
<td>0.019</td>
<td>0.87</td>
<td>4.4 ± 0.1</td>
<td>CLOSED</td>
</tr>
</tbody>
</table>

Figure 3.10: Experimental trajectories of 5 $\mu$m particles under the driving voltages of Table 3.1, showing good agreement with the predicted trajectories.

Collisions and hydrodynamic interactions certainly contribute, but any interaction causes perturbations of the trajectories, allowing the particles to re-enter the region of open streamlines and be transported away from the bubble. Importantly, this escape can only happen in a small region near the bubble pole (sketched in Fig. 3.11(c)), where (i) the relative particle velocities are large enough to provide strong perturbations, and (ii) the particles are not constrained by the gap. Escape thus happens at a well-defined position of the closed vortex loop (Fig. 3.11(b)), whereupon all particles have to follow a very similar open trajectory, resulting in focused bundles. To understand this process better, we simulate the flow field described by singularity theory (in Section 3.3). In this model, the flow field is Eulerian – the flow quantities are depicted as a function of position, $\mathbf{u}(x,t)$ (but steady streaming is steady state, i.e. $t$ independent). Particles are released far upstream from the bubble, and are followed through the Lagrangian flow field – particle velocity and positions are com-
puted and updated at each time step. In simulating the perturbations due to collisions and hydrodynamic interactions between the particles, we add velocity perturbations at every time step, with randomly chosen direction and a magnitude proportional to the local fluid velocity. Simulations applying random kicks to the particles reproduce this focused release process, in Fig. 3.11(c).

Figure 3.11: Releasing of $a_p = 5 \mu m$ particles (setup A, $f = 23.8$ kHz, $s \approx 0.015$, Poiseuille flow from right to left): (a) snapshot of accumulated $5 \mu m$ particle cluster; (b) streak image showing narrow trajectories of $5 \mu m$ particles (red) after releasing; (c) close-up of five simulated particle trajectories near the bubble surface, with small random perturbations applied at all times. All particles are trapped, then released by crossing the critical streamline in the indicated escape region (dashed box).

We demonstrate a general concept of non-invasive flow control through regulating bubble streaming strength relative to the Poiseuille flow. The flow domain is divided into regions of closed and open streamlines; particles can be trapped into or kicked out of closed-streamlines regions. This leads to separation of particles while they go through regions of maximum flow speed. The mechanism is different from the vast majority of passive size separation devices, where particles are typically steered to walls and thus separation occurs in the region of low flow speed [119–121].

3.5 Applications

Having understood the trapping and releasing mechanisms, we next show how to exploit them for applications. In the trapping mode, we can accumulate larger microparticles around the bubble and let the smaller particles pass by, effectively filtering different sized particles.
Preconcentration of microparticles: (a) snapshot of the 5-bubble set-up. The traces show the number of particles per time detected downstream of the last of five bubbles. The duty cycles are given by \((\tau_{on}; \tau_{off})\) values of (b) \((10s;10s)\); (c) \((20s;10s)\); (d) \((30s;10s)\).

In the continuous fashion, we can make use of the well defined trajectories of the released particles for *switching*, *sorting* different sized particles and constricting the spatial positions of the particles in the microchannel (known as *focusing*). Using oscillating microbubbles as basic actuating elements, we integrate them into various microfluidic devices to achieve different functions, demonstrating the versatility of microbubble streaming flows.

### 3.5.1 Preconcentrating/Filtering/Enrichment of microparticles

One direct application based on the trapping of selected-size particles is enrichment or pre-concentrating of these microparticles. Our goal here is to avoid release of trapped particles
and instead use the enrichment of particle concentration present in the clusters for purposes of preconcentration of the trapped species (and thus filtering with respect to undesired smaller species of particles). For this purpose, we use a programmable function generator to modulate the driving and excite the bubbles periodically, i.e. with a certain duty cycle. A multi-bubble set-up with a total of five microbubbles placed alternately on the sides of the main channel is used for this application.

During the times when the bubbles are excited, the bubbles trap clusters of particles. Upon deactivation of the bubbles, these trapped particles are released all at once and carried downstream by the Poiseuille flow. In this way, the particles are enriched and dispatched downstream periodically. Fig. 3.12 shows the number of particles per time going through a field of view downstream of the bubbles under different excitation cycles. The pattern is chosen as $(\tau_{on}; \tau_{off})$, where the ultrasound is turned on for the duration $\tau_{on}$, then remains off for $\tau_{off}$, and then the pattern repeats. Fig. 3.12 combines results of experiments with (10s;10s), (20s;10s), and (30s;10s). When the bubbles are not excited, the average number of particles per second is about 5. The release of the accumulated particles results in peaks of 10-40 times of this background average. The peak value increases with increasing time of trapping as more particles are trapped during the excitation cycle. By integrating more bubbles and increasing the trapping time, the enrichment can be further increased. Directly after the peak, for a duration of $\approx \tau_{off}$, a background signal which is from the concentration of normal solution can be observed. Enrichment of cells or other micron-sized objects can be accomplished in this fashion, yielding discrete clusters or bunches of particles localized both in $x$ (the downstream direction) and $z$ (the spanwise direction). After such preconcentration, the application of reactants or particle probes can be done much more efficiently [132]. As particles smaller than the critical size are passed through the device, a simultaneous filtering effect is also in evidence. By constricting the particles into thin bundles, this also serves as a solution to counteract Taylor dispersion [133].
3.5.2 Switching and sorting of microparticles

Upon escape from the upstream vortex (Fig. 3.11(c)), the selected-size particles follow a narrow bundle of trajectories downstream. This is of great interest in cell sorting and processing [20]. A desired sorting or switching of the bundles can be achieved easily in branched microfluidic devices. Here an H-shaped switching and sorting device is demonstrated (Fig. 3.13). It consists of two inlets for sample loading and two outlets for sample collection. Buffer solution without microparticles is injected into inlet $I_1$, whereas the solution containing particles is introduced into inlet $I_2$. A single microbubble is placed upstream of the junction. When the bubble is not excited, particles flow into outlet $O_2$. Upon excitation, the microbubble can trap and release particles, resulting in thin particle bundles. Depending on the relative strength parameter $s$, the thin bundles are then directed to the desired outlet. In Fig. 3.13(a)-(c) we show that the percentage of large particles released into two outlets can be tuned smoothly through changing $s$. When a mixture of different sized particles is introduced from inlet $I_2$, smaller and larger particles can be sorted to two different outlets, in Fig. 3.13(d). The device demonstrates good quality of separation for a mixture of 5 $\mu$m and 2.5 $\mu$m particles at $s \approx 0.013$: 100% of the large particles ($a_p = 5 \mu$m) exit at $O_2$, and 90% of the small particles ($a_p = 2.5 \mu$m) are transported to $O_1$.

Switching and sorting based on this concept can be extended to multiple-outlet or multi-level devices as well. Not only the switching can happen in a passive manner, but also an active decision by the user to steer particles to desired outlets is easily achieved. A three outlet switching device is shown in Fig. 3.14. In a practical scenario, users could simply divert various bio-particles/cells into a desired outlet for downstream processes by tuning the driving voltage.
Figure 3.13: Switching of 5 µm particles (setup B with particles injected in inlet $I_1$, $f = 20.8$ kHz): (a) bubble is not excited, all particles exit at $O_1$; (b) bubble streaming at $s \approx 0.024$ splits the particles evenly between $O_1$ and $O_2$; (c) stronger streaming ($s \approx 0.018$) diverts all particles to $O_2$; (d) sorting a mixture of 5 µm and 2.5 µm particles at $s \approx 0.013$: 100% of large particles exit at $O_2$, 90% of small particles at $O_1$.

Figure 3.14: Active switching of 5 µm particles with a three-outlets microfluidic devices, with increasing streaming flows (i.e. decreasing $s$) from (a) to (d).

3.5.3 Focusing of microparticles

Because of the narrow width of the released trajectory bundles, the trajectories that far upstream from a bubble span the interval $(0, h)$ in the $z$-direction are now confined to a much narrower interval after having been trapped. A simple modification to the setup can likewise focus those particles (at initial positions in the interval $(h, H)$) that have not been trapped in this first step: Positioning side channels (and thus bubbles) at equally-spaced
distances *alternatively* on the two walls of the main channel ensures that the entire width $H$ of the channel is subject to the trapping and focused release. The overall result is that the bubbles effectively force the particles to flow in a fraction of the channel, enforcing continuous concentration of particles in the $z$-direction. Fig. 3.15 shows streak images at different streaming strength, as well as the normalized histogram of particle position in $z$ across the channel width. In fact, for a given particle size, the focusing of particles happens in two stages: In the first stage (weaker streaming velocity, i.e., larger $s$), the particle size is smaller than the critical (gap) size, and particles are not trapped. Nevertheless, see Fig. 3.15(b), the bubbles compress the streamlines to a narrower region than the whole channel width to effect a limited focusing of the particles. In the second stage, $s$ becomes small enough for the particle size to be larger than $d_{gap}$: the particles are trapped and released into a narrow bundle of trajectories, see Fig. 3.15(f) and (g). The transition from the first stage to the second happens between Fig. 3.15(e) and Fig. 3.15(f) (i.e. $s$ between 0.052 and 0.041).

As is evident from the histograms, upon decreasing $s$ the focusing ability improves dramatically just before the trapping of particles sets in, with the eventual trajectory bundles reaching $z$-widths as small as $2\Delta z \approx 5.4 a_p$, where $\Delta z$ is the standard deviation of $z$-coordinates in the trajectory bundle exiting the field of view. Preconcentration or prepositioning of particles at this level is, again, very helpful for applications where e.g. cells are supposed to be transfected or tagged by contact with an agent that is only available or affordable in very small quantities. As $s$ decreases towards zero, the width $\Delta z$ is limited by the increased violence of particle-particle interactions (whose scale is set by the bubble streaming flow strength), which enables the release of particles from a wider region, thus widening the bundle of allowed escape trajectories. We observe an optimal $s$ of $\approx 0.05$ (in Table 3.2), which can be achieved through driving the piezoelectric transducer at $70 V_{rms}$ with a Poiseuille flow of $\bar{u}_p = 1.33 \text{ mm/s}$, well within the range of parameters accessible in our setup, as well as in a practical device.
Figure 3.15: Focusing of microparticles ($a_p = 5 \mu m$) in microchannel: (a) bubbles are not excited, $s = \infty$; (b) $s = 0.23$; (c) $s = 0.092$; (d) $s = 0.066$; (e) $s = 0.051$; (f) $s = 0.043$; (g) $s = 0.029$. 
Table 3.2: Experimental mean position and standard deviation $\Delta z$ of the microparticle trajectory bundles.

<table>
<thead>
<tr>
<th>$s$</th>
<th>$\infty$</th>
<th>0.24</th>
<th>0.094</th>
<th>0.068</th>
<th>0.052</th>
<th>0.041</th>
<th>0.034</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean position ($\mu m$)</td>
<td>116.6</td>
<td>119.6</td>
<td>122.2</td>
<td>125.4</td>
<td>136.7</td>
<td>164.2</td>
<td>184.3</td>
</tr>
<tr>
<td>standard deviation $\Delta z$ ($\mu m$)</td>
<td>50.6</td>
<td>50.6</td>
<td>29.2</td>
<td>18</td>
<td>13.5</td>
<td>18.1</td>
<td>18</td>
</tr>
</tbody>
</table>

3.5.4 Understanding of the focusing process

3.5.4.1 Experimental visualization of the flow field

A multiple bubble set-up is different from a single bubble set-up, because the streaming flows from neighboring bubbles may interact with each other (e.g. see Fig. 3.5(c)(d)). In order to better understand the focusing, we visualize the flow field by seeding both smaller and larger particles. Streak images produced from smaller particles give us the relevant information on the evolution of the flow portrait; while tracking of individual large particles allows a better understanding of the focusing process.

In Fig. 3.16(a)–(d), the streak images show the effect of $s$ on the overall appearance of the flow field with two bubbles located on separate walls. When bubble streaming is weaker (i.e. larger $s$), the bubbles only affect a locally small region, similar to that of a single bubble configuration (Fig. 3.7). The streamlines are slightly bent near the bubbles, but recover to a Poiseuille flow profile before going to the downstream bubble (Fig. 3.16(a),(b)).

As the bubble streaming strength increases (i.e. smaller $s$), the Poiseuille flow field is strongly affected by the bubbles. Part of the streamlines go through the gap between the bubble and upstream closed loop region; while part of the streamlines go above the bubble. Because of the staggered arrangement of the bubbles, the bubbles are able to constrict the streamlines from both sides of the main channel, (Fig. 3.16(a3)). With even stronger bubble streaming, some of the streamlines will go above the first bubble without going through the gap. However, these streamlines will enter the gap of the second bubble downstream. Together, the two bubbles make sure that all the large particles are trapped and released from the second bubble (Fig. 3.16(d)). Looking at trajectories of large particle in these
flow field (Fig. 3.16(a)–(d)), we see that \( s \) of optimal focusing is consistent with the earlier observation in the previous section 3.5.3.

Figure 3.16: Simultaneous visualization of flow field and large particle trajectories with a two bubble configuration with increasing streaming strength: (a) \( s \approx 0.24 \); (b) \( s \approx 0.16 \); (c) \( s \approx 0.11 \); (d) \( s \approx 0.04 \). The figures on the right panel show the corresponding particle \((a_p = 5 \, \mu m)\) trajectories.

3.5.4.2 Numerical simulation of focusing

We have also compared experimental results with numerical simulations to study the focusing process [100]. Briefly, in numerical simulations, the bubble streaming flow was modeled through Stokes flow singularities in the RNW formalism (described in section 3.3), and the Poiseuille flow superimposed through addition, consistent with the low-\( Re \) approximation of
this theory. The finite channel height, $H$, and multiple bubbles on either sides of the channel were simulated by the method of images [35, 127]. In modeling with singularity theory, we have simplified the flow field without considering the boundary layers near both solid wall and bubble interface. Additionally, we implicitly assumed that the flow is dipole-dominant at far field, because in Eq. (3.9) all the coefficients $C_n$ are $O(1)$ and the slow decaying term ($\psi_s(r, \theta, 1)$) dominates the far-field. While the experimental situation depicted in Fig. 3.15 is characterized by $Re_s = 0.6$, the RNW theory is strictly valid for $Re_s \ll 1$. Despite these shortcomings, the mechanism of focusing observed in the experiments can be understood using these simulations.

![Flow direction](image)

Figure 3.17: Snapshot of microparticle focusing inside a multi-bubble straight channel at $s \approx 0.05$: (a) experiment and (b) simulation.

To simulate random perturbations to the particles, we add velocity perturbations at every time step, with randomly chosen direction and a magnitude proportional to the local velocity. Hence, the velocity of a particle ($\mathbf{u}$) at a given time is given by $\mathbf{u} = \mathbf{u}_f + \alpha_p |\mathbf{u}_f| \hat{e}$, where, $\mathbf{u}_f$ is the unperturbed velocity of the particle, $\alpha_p$ is the strength of the perturbation and $\hat{e}$ is a unit vector pointed at a random angle. In our simulations, we have used two bubbles on opposite sides of the channel of height $H = 6.25a$ and separated by a distance $12a$. 

51
Approximately 50-60 particles all having radius \( a_p = 0.125a \) (corresponding to \( a_p = 5 \mu m \) particles in the experiments) and evenly distributed across the height of the channel were released one at a time from a distance of 5 bubble radii upstream of the first bubble. The only interactions considered between the particles themselves are the random velocity perturbations described above. The numerical algorithm also ensures that the impenetrability condition of the bubble surface is enforced by repositioning any particle colliding with the bubble. By using parameters from the experiment, the simulations capture qualitatively the focusing of microparticles flowing inside a straight microchannel, in Fig. 3.17.

Once the particles reach a distance of 15 radii downstream of the second bubble, the \( z \) coordinate values of the particles are used to compute the standard deviation \( \Delta z \). The variation of \( \Delta z \) with \( s \) is compared to that from the experiments in Fig. 3.18. The simulations capture the essential character of the \( \Delta z \) versus \( s \) curve which has a minimum for a non-zero \( s \), and is observed for a very similar value of \( s \) as in the experiments. We have chosen \( \alpha_p = 2 \) in our simulations to best illustrate the focusing mechanism. Varying \( \alpha_p \) around 2 only changes the magnitude of \( \Delta z \) but retains the value of optimum \( s \). This indicates that the effect of focusing is due to the intrinsic nature of the bubble streaming and does not significantly depend on the magnitude of the perturbations.

From our simulations we note that the criterion for optimum focusing is when all the particles hitting the first bubble also hit the second bubble and escape into the Poiseuille flow. This can occur if the critical streamline that passes close to the first bubble to within a distance of \( a_p \) also has the closest approach of \( a_p \) to the second bubble. From our computations we find that this condition is satisfied for \( s = 0.049 \), which is in very good agreement with the values of optimum \( s \) seen in the experiments and simulations (Fig. 3.18). Similar to the experimental observations, even in the simulations we see that below the optimum value of \( s \), the perturbations kick the particles into streamlines that diverge more and more with decreasing \( s \), and hence result in a decrease in the quality of focusing.
3.6 Conclusions

We have shown how to use microbubble streaming flows to manipulate microparticles in two qualitatively different ways to effect spatial concentration of the particles, for purposes of enrichment, switching and sorting, and focusing in microfluidic devices. Using the trapping abilities of the individual bubbles, we are able to accumulate the particles in discrete clusters around the bubbles, which could then be “read out” of the device as enriched bunches of enhanced particle concentration. Using the well-controlled release from trapping, we can switch and sort micro-sized objects by size. Additionally, we also find a continuous enrichment in the spanwise direction from the property of narrow bundling of particle escape trajectories. This process lacks focusing in the streamwise direction (or, equivalently, in time), but reaches much greater alignment in the spanwise direction, down to bundle widths on the order of the particle diameter. In this fashion, the second method of continuous focusing is also potentially applicable for particle positioning, as the position of the bundle changes with the flow parameters.
The applications demonstrated in the present chapter highlight the importance of characteristic flow geometry for particle manipulation, a general principle behind bubble microstreaming flows: not only does the location of the critical streamline (Fig. 3.7) determine the critical particle size for trapping, but the setup with alternating bubbles on both sides of the main channel relies on the location of the respective critical streamlines for the desired focusing effect. As the streamline portrait can be easily and quickly changed through changes in ultrasound driving amplitude and/or frequency, interactive modification of the setup is an additional feature of such devices, adding an active component to control on top of the passive driving of the bubble-induced flow. We have demonstrated one instance of such interactive control through particle enrichment (where the control is a selection of “on” and “off” duty cycles), but a variety of more sophisticated control patterns can be envisioned. Bubble microstreaming thus offers a versatile toolbox, whether focusing on a simple passive device with no need for feedback, or on an active device with feedback for immediate selection of a desired flow.

Only acoustically driven microbubbles provide both sufficiently high amplitudes of oscillation for steady streaming and the desired geometric confinement to a well-defined gap – streaming from solid objects [134,135] is too weak on such small scales, while flexural plate wave (FPW) streaming [136] or AC electrophoresis [106] does not provide the gap geometry. The flows do not rely on density differences, large particle Reynolds numbers [137] or on dissipative/radiation force effects [116,118], thus incurring negligible heating of the fluid. The latter is desirable for handling biological objects such as cells in cytometry, just one of many potential applications for this general method.
Chapter 4

Frequency dependent bubble dynamics and bubble streaming flows

We have demonstrated that microbubble steady streaming is a powerful actuating mechanism in microfluidics for micro-sized object manipulation in the frequency range of $10 \text{kHz} < f < 30 \text{kHz}$ by utilizing the most generic type of streaming flows – two vortices drawing liquid towards the bubble. In experiment, we have observed a variety of flow patterns while changing driving frequency $f$ between 1 kHz and 100 kHz. The frequency dependence has prompted us to look deeper into the fundamentals of bubble streaming, as we realized that there was neither enough quantitative data nor a satisfactory theory to describe the experimentally relevant situation. Therefore the objective of this chapter\(^1\) is to develop both – quantitative experimental measurement as well as a theoretical understanding of the observed phenomenon. To understand such frequency dependence, we experimentally characterize the oscillation modes and the frequency response spectrum of acoustically driven microbubbles over this frequency range. We find that (i) the appearance of streaming flow patterns is governed by the relative amplitudes of bubble surface modes (normalized by the volume response), (ii) distinct, robust resonance patterns occur independent of details of the set-up, and (iii) the experimental results compare well with the prediction of an asymptotic theory. At lower frequencies, where there are significant contributions by individual surface modes, the flow patterns are dominated by mixed mode streaming from the bubble. At higher frequencies, where the oscillatory flow is dominated by the monopole mode, streaming along the wall becomes important. Lastly, we also examine the effect of several geometric factors – bubble shape, aspect ratio of the confined bubble $D/w$, and second wall separation distance

\(^1\)This chapter is adapted from part of Ref. \cite{5, 138}.
4.1 Introduction

4.1.1 Acoustic manipulation of fluid

The use of acoustic waves ranging from audible frequency to ultrasound in microfluidic environments (denoted by the term *acoustofluidics*) has enabled versatile manipulation of fluid, as well as of micro/nano-sized objects such as particles, bubbles, and cells [12]. Ultrasound standing waves in the MHz range have been used to trap and separate cell/particles [139] through acoustic radiation forces, which can move the suspended particles/cells to different lateral positions within a laminar stream. Surface acoustic waves (SAWs) of order 100 MHz, which actuate on the fluid as a whole, have demonstrated several practical applications as well, with examples including cell and droplet sorting [118, 140], free surface liquid pumping [141], and concentration particles [117]. The operation frequency range of the above mentioned methods must be high enough to have acoustic wavelengths comparable to the length scale of cell/particle or microdevices, or to generate momentum flux to move the liquid directly. The potentially undesirable effects are the relatively high power consumption and temperature rise due to heat generation, which may be a concern for biological samples sensitive to temperature. In addition, they both require precise fabrication, such as arrangements of inter-digitated structures [117, 118, 140].

Acoustic streaming, a classical phenomenon of driving fluid using sound, has found many useful applications at the microscale over the last decade. As pointed out by other researchers [12, 63, 142], a distinction must be made between two general types of streaming: one being a result of attenuation of energy into the fluid during sound propagation (e.g. “quartz wind”), and the second being due to the Reynolds stresses within a thin boundary layer (known as boundary induced *steady streaming*). The former type of acoustic streaming needs to operate in the MHz range to drive steady currents in water [136]. By contrast, boundary steady
Streaming can be used at a much lower driving frequency to generate steady flows [35, 143]. While it is possible to integrate vibrating suspended microstructures [135, 144] into a microdevice to induce streaming, such an approach is usually very complex in microfabrication. An alternative way is to induce fluid oscillation over solid objects [134, 145], which is often limited by the low oscillation frequency and small streaming velocity.

### 4.1.2 Acoustic bubble streaming

Ultrasound-driven oscillating microbubbles serve as an excellent actuator to induce microscale steady streaming, offering several advantages such as simple manufacture, easy integration into microfluidic system, and large oscillatory amplitude and thus larger streaming velocity. Protruding air pockets can form spontaneously from indentations in 3D [33–36] or from blind side channels in 2D setups [40–43], see Fig. 4.1. A commercially available piezoelectric transducer can be easily glued anywhere on the substrate to provide excitation, as the direction of acoustic waves is immaterial, in contrast to standing wave or SAW techniques. The compressibility of the bubble enables large-amplitude oscillations of the bubble interface ($\varepsilon a$ with $\varepsilon = 0.05$, where $a$ is the bubble radius). In the last few years, many microfluidic applications based on bubble streaming have been developed, including mixing enhancement [40, 146], particle sorting and switching [41, 42], and particle focusing and enrichment [43].

However, a fundamental understanding of microbubble streaming flow lags behind experimental progress. While general theories exist for streaming induced by oscillatory flow over no-slip surfaces [147], or for bubble-induced streaming in bulk fluid [65, 148], the particular situation in practical devices (Fig. 4.1) is complicated by the combination of (i) the no-stress bubble boundary condition, (ii) the deformability of the bubble interface, (iii) the contact line between the bubble interface and the wall, and (iv) the necessity of matching the oscillatory boundary layers around the bubble and at the wall. Elder [62] in his pioneering work already described the rich and complex flow patterns from a 3D oscillat-
ing hemi-spherical bubble attached to a wall, submerged in liquids of different viscosities. Tho et al. reported experimental investigations of flow fields from 3D hemi-spherical bubbles confined between two plane walls [149]; in this geometry, qualitative and semi-quantitative descriptions emerged [36, 37], but without accounting for the problems (iii) and (iv) above. In contrast to these studies, microfluidic devices often have 2D planar geometry due to the lithography-based microfabrication technique, so that 2D microbubbles (menisci) are more commonly encountered in practical microfluidics applications [40–43, 150].

Here, we study the oscillations of a 2D oscillating bubble sandwiched between two plane walls (Fig. 4.1) and attached to a solid side wall under different driving frequencies, as well as the flow patterns arising from the bubble dynamics. The secondary steady streaming flow is the time-averaged result of the first order oscillatory flows, which in turn are caused by the oscillating bubble. To measure the bubble dynamics quantitatively, we use high-speed imaging at up to 100,000 frames per second to resolve the bubble interface shape in space and time.

4.2 Materials and methods

4.2.1 Experiment set-up

The experimental set-up is similar to the ones previously described 2.2 and 5.2.1, schematically shown in Fig. 4.1. The microfluidic channel is made of Polydimethylsiloxane (PDMS) using soft lithography [87]. The microfluidic device is then bonded at the substrate slide (either glass or polystyrene) after treatment with oxygen plasma. The microfluidic device has a main channel with a depth of \( D = 100 \mu\text{m} \) and height in the image plane of \( H = 1000 \mu\text{m} \), and a side channel with a opening of \( w \approx 80 \mu\text{m} \) wide (Fig. 4.1(b)).

When introducing aqueous glycerol solution (23% glycerol by weight) into the main channel through a syringe pump (PHD Ultra, Harvard Apparatus), an air bubble close to semi-cylindrical shape protruding into the main channel forms in the side channel Fig. 4.1(c)). A
Figure 4.1: Schematic of the experimental set-up for bubble dynamics measurement (not to scale): (a) and (b) show the side view and top view; (c) a perspective view of the semi-cylindrical bubble; (d) a snapshot of the undisturbed bubble (scale bar is 50 µm); (e) the coordinate system used to measure the bubble shape.

piezoelectric transducer (thickness 1 mm, diameter 10 mm, Physik Instrumente, Germany) glued to the glass slide provides ultrasonic excitation of the bubble, using sinusoidal signals of frequency $f = 1 - 100$ kHz from a function generator (7075, Hioki, Japan) and amplifier (7500, Krohn-Hite, USA). The device is illuminated by a halogen source (TH4-100, Olympus, USA) for transmitted-light bright-field microscopy.

We use an inverted microscope (IX71, Olympus) with a 20× or 40× objective lens, together with a high-speed camera (Phantom v310, Vision Research, USA) to capture top-view images. Polystyrene microparticles of radii $a_p = 0.5 - 1$ µm (Magsphere Inc) are suspended in a density-matched water-glycerol solution as tracers for streak visualization. Streak photographs are obtained by superposing a series of typically 1000 successive images at a frame rate of 1000 frames per second (fps). To study the dynamics of the bubble interface, we capture images of $120 \times 80$ pixels, at 100,000 fps and with an exposure time of 1 µs (Fig. 4.1(d)).
4.2.2 Data analysis

We vary the driving frequency $f$ from 1.6 kHz to 103.6 kHz, with an increment of $\Delta f = 0.5$ kHz between 1.6 kHz and 25.6 kHz and of $\Delta f = 1$ kHz between 25.6 kHz and 103.6 kHz, while keeping the input driving voltage to the piezoelectric transducer constant. At each frequency, a total of 1000 consecutive images are recorded. The recorded images are imported to freeware ImageJ (NIH, USA) [90], first converted to binary images with proper thresholding, and then the bubble outline in each frame is extracted and saved. We use MATLAB to characterize the bubble outline as a radial function $r(\theta, t)$, measured in polar coordinates from an origin at the center of the side channel opening, as shown in Fig. 4.1(e).

We will show that information about the bubble motion in the radial-azimuthal plane (Fig. 4.1(e)) is sufficient to explain its behavior, i.e., oscillations in the direction of the axis of the cylindrical bubble have negligible effect and both the bubble surface oscillations and the resulting flow fields can be understood as two-dimensional dynamics in the radial-azimuthal plane. Below, we present both experimental evidence and theoretical justification for this treatment of the problem as an oscillating 2D bubble.

The shape of the initially undisturbed bubble is described by $r_0(\theta, 0)$. The angular-dependent amplitude of the bubble is then characterized by $\Delta r(\theta, t) = r(\theta, t) - r_0(\theta, 0)$. Even though the camera sampling rate is almost the same as the higher driving frequencies $f \sim 100$ kHz, the very short exposure time (1 $\mu$s) and carefully chosen driving frequencies allow us to improve the time resolution using stroboscopic techniques (see sec. 2.4 for details). For each frequency, we use the middle 500 frames of the captured images for analysis. We then determine the mode amplitude and phase angle by performing Fourier decomposition according to

$$\Delta r(\theta, t) = a \sum_{n=0} a_n \cos(2n\theta) \sin(\omega t + \phi_n),$$  \hspace{1cm} (4.1)

where $\omega = 2\pi f$ is the angular frequency, $a_n$ are the dimensionless mode amplitudes, and $\phi_n$ the phase angles. The decomposition into cosines is suggested by the symmetry of the
4.3 Correlation between bubble dynamics and streaming flow patterns

In this section, we will focus on understanding the correlation between the flow patterns of secondary steady streaming and the fast time-scale oscillations of the microbubbles. Through high-speed imaging analysis, we decompose bubble motion into different shape modes and determine their amplitudes and phases. With these quantities, we compute the relative streaming strength resulted from different modes and establish the link between the streaming flow patterns and the mixed-mode streaming of the microbubble. Both the bubble dynamics and the streaming flow patterns are explained and compared to the prediction of an asymptotic theory.

4.3.1 Flow patterns at different driving frequency

When changing driving frequency $f$ in the range of $1.6 \text{ kHz}$ to over $100 \text{ kHz}$, we have observed a succession of different flow patterns, as shown in Fig. 4.2. At lower frequencies (Fig. 4.2(a)-(b)), there are two symmetric vortices above the bubble, drawing liquid towards the bubble
and pushing liquid upwards along the pole of the bubble. We denote this pair as “fountain” vortices or loops. This flow pattern has been described as the “generic” streaming pattern near an interface, both in the present 2D set-up [40,42] and (as an analogous toroidal vortex loop) for 3D hemispherical bubbles [35,36,62]. As $f$ increases in our set-up, a second pair of vortices is observed to appear, with orientation opposite (“anti-fountain” to the first pair (see Fig. 4.2(c)). With even higher driving frequencies, the “anti-fountain” vortices dominate over the “fountain” vortices (see Fig. 4.2(d)), reversing the far-field flow pattern. In Fig. 4.2(e)-(h), we show the corresponding outlines of the bubble movement by superimposing high-speed images over one cycle near the frequencies of the streak images (of Fig. 4.2(a)-(d). At frequency $f \approx 10$ kHz, the outline shows a single crescent (antinode) near the pole of the bubble. With increased $f$, more nodes and anti-nodes appear, see (f) and (g). At even higher $f$, the outline seems to have a uniform oscillation along the entire bubble. The different node patterns indicate the presence of frequency-dependent bubble oscillation (shape) modes. For a free bubble driven by a time-varying pressure field, the monopole is excited most effectively and separately from any shape modes. For a bubble located at the side channel opening, however, shape and volume modes have to be excited together to accommodate pinning of the contact line. Note that this pinning may be weak: There may be some mobility of the contact line, but because it is located near the corner connecting the horizontal wall and the side channel wall, its location changes little even for significant bubble oscillations. In the next subsection, we present quantitative measurements of these mode contributions.

4.3.2 Two dimensional character of bubble dynamics

In many microfluidic devices, as in ours, the bubble is confined by two parallel supporting walls to which it is attached via large contact areas (in Fig. 4.3(f)), thus assuming approximately cylindrical symmetry. Experimentally we have also verified that these contact areas are immobile. Now we will first present experimental evidence to show that these flow patterns change little in the direction perpendicular to the views presented, i.e., these flows
Figure 4.3: Streak images at different depth $y$ show similar streaming flow patterns ($f = 16.8$ kHz). (a) middle of the bubble, $y = 0$, (b) $y = 10 \mu$m, (c) $y = 20 \mu$m, (d) $y = 30 \mu$m, (e) $y = 40 \mu$m, and (f) near the top wall $y = 50 \mu$m.

Figure 4.4: (a) Streaming velocity $\bar{u}_s \equiv u_s(y)/u_s(y = 0)$ and (b) oscillating amplitude $\bar{\epsilon} \equiv \epsilon(y)/\epsilon(y = 0)$ at different depth $y$. 
have two-dimensional characteristics. Here we record particle motions at imaging planes at five different depths from the channel center \( y = 0 \). By superposing the successive images, we obtain the streak photographs. As shown in Fig. 4.3, the streak images at different depth \( y \) exhibit similar streaming flow patterns. To further quantify the oscillating amplitude (i.e. \( \epsilon(y) \)) at different \( y \), we can estimate \( \epsilon(y) \) as follows. Recall that streaming velocity \( u_s(y) \sim \epsilon(y)^2 a(y) \omega \), the dimensionless oscillating amplitude is therefore

\[
\epsilon(y) \sim \left( \frac{u_s(y)}{a(y) \omega} \right)^{1/2}.
\] (4.2)

Using the particle tracking method, we measure steady streaming velocities in three regions at those five different imaging planes. We define \( \bar{u}_s \equiv \frac{u_s(y)}{u_s(y = 0)} \) as a measure of the velocity variation at different depth \( y \). By examining the bubble outlines in Fig. 4.3, we can determine the bubble radius \( a(y) \) with imaging analysis and curve fitting a circle: at the middle plane, \( a(y = 0 \mu m) = 42.5 \mu m \), and near the top wall, \( a(y = 50 \mu m) = 37.5 \mu m \). We can already see that the bubble shape approximates as a half cylindrical bubble sandwiched between the two walls in the \( x-z \) plane. Although the exact variation of the bubble radius along \( y \) is unknown, we can assume a simple parabolic variation of bubble radius such that the radius is maximum at \( y = 0 \). With Eq. (4.2), and defining \( \bar{\epsilon} \equiv \frac{\epsilon(y)}{\epsilon(y = 0)} \), we can therefore plot both the variation of streaming velocity and the dimensionless oscillation amplitude as a function of depth \( y \) in Fig. 4.4. Both streaming velocity and oscillating amplitude decrease slowly over a large portion of the channel depth and only decay to zero rapidly near the top sandwiched wall (\( y = 50 \mu m \)). This suggests that the bubble oscillations are approximately two-dimensional in the radial-azimuthal (or \( x-z \)) plane, and so are the resulting steady streaming flows. This experimental evidence also justifies the use of 2D theory [5] to describe both the bubble dynamics and the streaming flows. Additionally, the theory shows that the expected excitation amplitude of the axial oscillation modes decays exponentially with distance away from the bubble surface and is significantly smaller than
that of the azimuthal modes [5].

### 4.3.3 Mode amplitude and phase

Now we decompose the bubble outline in the $x$-$z$ plane with Fourier analysis of Eq. (4.1) to determine both the amplitude and phase of the shape modes. We plot the amplitude of the first four ($n = 0, 1, 2, 3$) modes for a microfluidic device mounted on a glass substrate in Fig. 4.5(a); Fig. 4.5(c) shows the difference of phase angles between the shape modes $n > 0$ and the volume mode $n = 0$, through the term $\sin(\phi_n - \phi_0)$. The bubble amplitude response curves in Fig. 4.5(a) are jagged, while the phase difference shows a smooth change as $f$ is
varied (Fig. 4.5(c)). The strong variations in the amplitude spectrum can be explained by the actual driving pressure levels acting on the bubble, which depend on both the characteristic resonances of the piezoelectric transducer and the characteristic response of the entire fluidic set-up, changing with its material composition and geometry. In an earlier experimental study, Tho et al. measured the pressure level using a hydrophone and found a strongly nonlinear acoustic pressure amplitude as a function of excitation frequency [149]. In other words, the non-smooth amplitude curve is attributed to a non-constant driving pressure, though the driving voltage to the piezoelectric transducer is kept constant.

We demonstrate the dependence on material composition by measuring the bubble response with a different set-up where the substrate slide is made of polystyrene. Here, instead of bonding the PDMS fluidic device onto a glass slide, we bond it onto a polystyrene slide when making the top substrate (refer Fig. 2.2 for assembly of the device). Then the top substrate is then mounted onto the same bottom glass substrate, so the assembled device consists of a polystyrene and a glass substrates. With this change of substrate material, the details of the resulting amplitude spectra (Fig. 4.5b) are very different from that of a all-glass substrate device, Fig. 4.5(a). By contrast, when comparing the measured phase angles Fig. 4.5(d) and Fig. 4.5(c), we observe almost identical phase spectra, indicating these as intrinsic characteristics of the bubble.

Encouraged by the universal spectra of relative phases, we proceed to analyze the analogous amplitude property, i.e., the relative amplitude of each mode normalized by that of the volume mode, \( \frac{a_n}{a_0} \). In Fig. 4.6(a), \( \frac{a_n}{a_0} \) is plotted for both the microfluidic devices mounted on the glass substrate and the polystyrene substrate, respectively. The curves are in very close agreement, indicating that the quantity \( \frac{a_n}{a_0} \) can be used to describe the intrinsic bubble response regardless of the fluidic set-ups. This normalization strategy thus allows for meaningful comparison of data across set-ups and reveals a much simpler, robust resonance structure. We note that the monopole amplitude is always the largest for all driving frequencies, and to a first order approximation the monopole amplitude sets the dimensionless
Figure 4.6: Normalized mode amplitudes $\bar{a}_n$ with respect to monopole (a); and relative streaming strength $I_n$ (b). Open symbols are for the glass substrate, filled symbols for the polystyrene substrate.

amplitude ($\epsilon$) scale, although higher order amplitudes $a_n$ contribute to $\epsilon$ as well.

The higher order modes show distinct peaks, around 12 kHz for $n = 1$, 30 kHz for $n = 2$, and 45 kHz for $n = 3$ respectively. However, it must be pointed out that these peaks are not conventional resonant frequencies, which are defined as peaks under a constant driving pressure (note that direct measurement of driving pressure with hydrophones is possible in cm-scale fluid chambers [149], but is impractical inside a sub-mm microchannel). The relative amplitudes and phases thus emerge as important parameters to characterize the bubble behavior and subsequently predict the streaming flow, as discussed below.

With the relative mode amplitudes known, we can determine the first order oscillating velocity magnitude as well as the kinetic energy of each mode, $u_n \propto \bar{a}_n$ and $E_n \propto u_n^2 \propto \bar{a}_n^2$. The percentage of power contained in the individual mode is therefore,

$$P_n = \frac{E_n}{\sum_{i=0}^{N} E_i} = \frac{\bar{a}_n^2}{\sum_{i=0}^{N} \bar{a}_i^2},$$

(4.3)

where $N$ is the total number of modes present the oscillation. In the analysis here, we show
the mode number up to $N = 3$, as higher modes are only excited at higher frequency and generally with smaller amplitude compared to the monopole. Fig. 4.7 shows the percentage of power contained in each modes. The dominance of monopole is clear throughout the frequency range analyzed, between 5 to 100 kHz. In particular, at higher frequency ($f > 60$ kHz), more than 90% of the power is contained in the monopole.

### 4.3.4 Relative streaming strength and flow patterns

Steady streaming through Reynolds stresses at boundary layers can always be interpreted as a second-order effect in the amplitudes of first-order oscillatory flows, i.e., in our case, steady streaming flow components are proportional to quadratic terms of mode amplitudes. Nominally, one would expect the term $\propto a_0^2$ to be the strongest contribution on account of the large $a_0$ values, but a pure (radial) volume oscillation does not lead to any steady streaming [65]. Therefore, the dominant streaming terms should be those resulting from the interaction of the volume mode and an $n > 0$ shape mode. The velocity scale of this mixed-mode streaming can be shown to be proportional to $a_0 a_n \sin(\phi_n - \phi_0)$, i.e., the phase
shift between the modes is important. Making use instead of our normalized amplitudes, we conclude that $I_n \equiv \bar{a}_0 \bar{a}_n \sin(\phi_n - \phi_0)$ is a measure of bubble streaming intensity due to the interaction between higher order and monopole oscillatory flows.

We plot $I_n$ for the microfluidic devices mounted on glass and polystyrene substrates in the same graph, Fig. 4.6(b). The data from both set-ups again coincide, indicating streaming intensity as an intrinsic property of the bubble of a fixed radius. A resonance spectrum is observed: as $f$ is increased, $I_1$ has the largest magnitude from between a few kHz to about 25 kHz and peaks around 15 kHz. Between 25 kHz and 40 kHz, $I_2$ is seen to have larger a contribution, with a peak at about 30 kHz, while $I_3$ peaks around 50 kHz. The streaming flow depends on other modes of streaming as well, for example contributions like $\bar{a}_1 \bar{a}_2 \sin(\phi_2 - \phi_1)$. However, these are typically small, because the prefactors are small (e.g. $\bar{a}_1 \bar{a}_2 \sin(\phi_2 - \phi_1) \lesssim 0.2$), and also because higher-order streaming contributions decay faster.
with distance from the bubble.

In general, higher order mixed streaming tends to have more complex flow structure near the bubble surface. In order to visualize the finer flow structures of the streaming flow, particularly near the bubble surface, we use smaller tracers \( a_p = 0.5 \mu m \) and a 40× objective lens. Streak photographs of four different \( f \) are shown in Fig. 4.8. A closer examination of Fig. 4.6b and Fig. 4.8 shows a change of flow patterns with varying prominence of different \( I_n \). At low frequency in Fig. 4.8(a), the predominant two “fountain” vortices flow structure is a result of the streaming of \( n = 0 \) and \( n = 1 \) mode. As \( f \) is increased to around 30 kHz, a second pair of small “anti-fountain” appears near the pole, a result of stronger \( n = 0 \) and \( n = 2 \) mode streaming (Fig. 4.8(b)). Further increase of \( f \) induces even more complex structures near the bubble surface, and at the same time another pair of “anti-fountain” vortices emerges from the wall (Fig. 4.8(c)). At the highest \( f \), the flow near the bubble has many small vortices (between the dashed line and the bubble surface), while the “anti-fountain” near the wall grows larger and dominates the whole flow field, see Fig. 4.8(d).

4.4 Comparison with asymptotic theory

4.4.1 Bubble dynamics

The resonance features of the bubble oscillation can be understood within an asymptotic theory framework that accounts for the coupling of azimuthal modes through the boundary conditions. We briefly describe how the theory works, while detailed theory can be found in Ref. [5]. In the theoretical model, we consider an idealization of the experimental set-up: a cylindrical bubble of radius \( a \) confined between parallel plates of distance \( D \), and pinned to a wall by means of two contact lines. Note this idealization is reasonable considering the actual shape we have observed in experiment.

First, the oscillatory velocity field is constructed using an asymptotic matching method to satisfy the kinematic boundary condition at the bubble interface as well as the no-slip
boundary condition at the solid boundary wall. Such a velocity field will depend on the shape of the oscillating bubble – both on volume and surface modes. With the solution of the oscillatory velocity field constructed, the pressure field and the viscous stress can then be found. At the bubble interface, normal stress balance between the pressure, viscous stress and the surface tension stress gives the shape of the bubble. Decomposing the bubble shape into a Fourier series like experiment allows a direct comparison between the experiment and theory, in Fig. 4.9. The peaks of the relative amplitude curves occur near the resonance frequencies for the a bubble in free space \( \Omega_n = \Omega \sqrt{2n(4n^2 - 1)} \) \([138]\), where \( \Omega = \sqrt{\Gamma/\rho a^3} \) and \( \Gamma \) is the surface tension coefficient of the interface. The main features of the amplitude and phase curves are reproduced consistently for each surface mode and are a consequence of the surface mode coupling to the volume mode as well as the coupling of pairs of surface modes to each other.

\[
\Omega_n = \Omega \sqrt{2n(4n^2 - 1)}
\]

\[\Omega = \sqrt{\Gamma/\rho a^3}\]

Figure 4.9: (a) Relative amplitudes \( \bar{a}_n \) and (b) sine of phase angles \( \phi_n \) of the first three even surface modes. The markers correspond to experimentally measured values for a nearly semi-cylindrical bubble, and the solid lines are predicted by the theory. The dotted lines in (a) indicate the undamped resonance frequencies of corresponding surface modes of free cylindrical bubbles.
4.4.2 Steady streaming flows

Previously (sec. 3.3), we have seen that RNW singularity theory predicts the steady streaming flows qualitatively and serves as a convenient way for computation and guiding fluidic device design, particularly useful for streaming flows in the frequency range of 10 – 30 kHz. Now with a better understanding of the frequency dependent bubble dynamics over a wide frequency range, we are able to develop a more sophisticated theory – describing the steady flow patterns from first principle fluid dynamics. We will outline the general procedure of the theoretical treatment, important governing equations as well as the boundary conditions, while the details can be found in Ref. [138].

The asymptotic approach involves solving both the leading order oscillatory and the second order steady flow fields, by applying proper kinetic boundary conditions at the walls and the bubble interface. The leading order oscillating flow field, $\psi_0$, is described as

\[
\left( \frac{2}{\delta^2} \frac{\partial}{\partial t} - \nabla^2 \right) \nabla^2 \psi_0 = 0. \tag{4.4}
\]

The dynamics of the bubble surface (known from either experimental measurement or asymptotic theory) is described in units of $a$ as,

\[
R(\theta, t) = 1 - i\epsilon\zeta(\theta)e^{it} \tag{4.5}
\]

where $\zeta(\theta)$ is $O(1)$ and only the real part of any complex quantity is physically meaningful. The boundary conditions at the mean position of the interface is therefore [138],

\[
\begin{aligned}
\frac{1}{r} \frac{\partial \psi_0}{\partial \theta} &= \zeta e^{it} & \text{on} & & r = 1, \\
\frac{\partial^2 \psi_0}{\partial r^2} - \frac{1}{r} \frac{\partial \psi_0}{\partial r} - \frac{1}{r^2} \frac{\partial^2 \psi_0}{\partial \theta^2} &= 0 & \text{on} & & r = 1,
\end{aligned} \tag{4.6}
\]
in addition to no-slip conditions at the walls,

\[
\frac{\partial \psi_0}{\partial r} = \frac{1}{r} \frac{\partial \psi_0}{\partial \theta} = 0 \quad \text{on} \quad \theta = 0 \text{ and } \theta = \pi .
\] (4.7)

Solution of the first order oscillatory flow field provides the forcing term that drives the second order steady streaming flow. The steady streaming flow is described by the following Stokes equation,

\[
\nabla^4 \langle \psi_1 \rangle = -\frac{2}{\delta^2} \left\langle \frac{1}{r} \frac{\partial (\psi_0, \nabla^2 \psi_0)}{\partial (r, \theta)} \right\rangle .
\] (4.8)

To properly account for the drift due to the first order oscillatory flow, the steady motion of individual fluid elements needs to be evaluated by augmenting the Eulerian stream function with a Stokes drift term \( \psi_d \), defined as [138, 151]

\[
\psi_d = \left\langle \frac{1}{r} \frac{\partial \psi_0}{\partial \theta} \int -\frac{\partial \psi_0}{\partial r} \, dt \right\rangle .
\] (4.9)

The Lagrangian stream function is then \( \Psi = \langle \psi_1 \rangle + \psi_d \). For \( \Psi \), both radial velocity and tangential stress vanish at the mean position of the interface [138], i.e.,

\[
\begin{aligned}
\frac{1}{r} \frac{\partial \Psi}{\partial \theta} &= 0 \quad \text{on} \quad r = 1 , \\
\frac{\partial^2 \Psi}{\partial r^2} - \frac{1}{r} \frac{\partial \Psi}{\partial r} - \frac{1}{r^2} \frac{\partial^2 \Psi}{\partial \theta^2} &= 0 \quad \text{on} \quad r = 1 ,
\end{aligned}
\] (4.10)

in addition to satisfying no-slip conditions at the walls, given by

\[
\frac{\partial \Psi}{\partial r} = \frac{1}{r} \frac{\partial \Psi}{\partial \theta} = 0 , \quad \text{on} \quad \theta = 0 \text{ and } \theta = \pi .
\] (4.11)

There exist boundary layers near both the walls and the bubble interface due to the first order oscillating flow field, so we will need to replace the no-slip conditions Eq. (4.11) with a slip velocity \( U_s \) as the matching boundary condition to the bulk outside the boundary layers. For an imposed oscillatory slip velocity \( u_s(r)e^{it} \), the steady Lagrangian slip velocity
that persists at the outer edge of the wall boundary layer is given by [138,152],

\[ U_s = -\frac{3 - 5i}{4} u_s^* \frac{du_s}{dr}, \]

(4.12)

where \( u_s^* \) is the complex conjugate of \( u_s \).

After significant algebraic manipulation, a consistent solution can be found for the Lagrangian stream function fulfilling the governing equations and boundary conditions at the bubble interface and the wall (see Ref. [138] for details of the derivation). The final solution for \( \Psi \) can be written as [138],

\[ \Psi = \sum_{k=1}^{\infty} e_k \frac{1}{r^{2k-1}} \left\{ \cos(2k-1)\theta - \cos(2k+1)\theta \right\} + \sum_{k=1}^{\infty} f_k \frac{1}{r^{2k}} \left\{ \frac{1}{2k} \sin 2k\theta - \frac{1}{2(k+1)} \sin 2(k+1)\theta \right\} + \psi_s + \Psi^-, \]

(4.13)

where \( e_k \) and \( f_k \) are coefficients of two series of no-slip Stokes solutions, and \( \psi_s \) represents homogeneous slip solutions of (4.8), given by

\[ \psi_s = \sum_{m \geq n} \sum_{n=0}^{\infty} \frac{\bar{a}_m a_n}{r^{2(m+n+1)}} \frac{3}{4} \left\{ \cos \phi_{m,n} + \frac{(m-n)\sin \phi_{m,n}}{m+n+1} \right\} \sin 2(m+n+1)\theta, \]

(4.14)

and

\[ \Psi^- = \bar{a}_m a_n \left\{ \frac{1}{2r^{2(m+n+1)}} + 2i \delta^2(2m+1)(2n+1) e^{-(1+i)\eta} \right\} \sin \phi_{m,n}. \]

(4.15)

The coefficients \( e_k \) and \( f_k \) are analytically known, and are expressed as functions of \( \psi_s \) and \( \Psi^- \), by applying the boundary conditions (4.10) at the surface of the bubble to the Lagrangian steady stream function in (4.13). With the closed solution Eq. (4.13), we only need to know the \( \bar{a}_n \) and \( \phi_{m,n} \) to explicitly evaluate the flow fields, and these in turn follow from the oscillator theory simply with the driving frequency and other experimental parameters [5] as input.
4.4.3 **Comparison of flow patterns and velocity**

We evaluate the steady streaming using mode amplitudes and phases both from experimental measurements and dynamical calculations, which are in good agreement with each other. We find that the steady flow pattern is characterized by closed vortical flow lines. Over a wide range of frequencies and damping parameters, the velocity field in the bulk of the fluid is inward close to the wall, and radially outwards near the pole of the bubble in a “fountain” vortex pair, precisely the generically observed flow pattern of bubble microstreaming devices, see Fig. 4.2 and Fig. 4.10(a)(b). A tiny secondary vortex pair near the pole of the bubble is also typically predicted and observed in experiment (Fig. 4.10(a)(b)).

![Figure 4.10: Comparison of fountain flows in experiment and theory. (a) Experimental streamlines of Lagrangian steady flow at f = 26.7 kHz; (b) computed streaming pattern at the corresponding dimensionless frequency λ = 9.32, using amplitudes and phases from the analysis of [5]. The agreement is representative of the entire regime of fountain flow patterns. (c) Steady Lagrangian azimuthal velocity v along lines of zero radial velocity (indicated as dot-dashed line in (b)), as a function of radial distance r: direct measurements from an experimental run at f = 26.7 kHz (○), computed from bubble oscillation amplitudes obtained from interface tracking experiments [5] of a different run at the same f (−−), and computed from theoretical bubble oscillation amplitudes using only λ = 9.32 as input (---).](image)

To compare not just the flow patterns, but the observed velocities, we evaluate the azimuthal velocity along a line through the points of zero radial velocity in one of the vortices (Fig. 4.10(b)). From experimental movies, we determine the amplitude factor ε as the half-distance between maximum and minimum radii of bubble oscillation (for Fig. 4.10(c),
Figure 4.11: Comparison of anti-fountain flow pattern from experiment and theory at high frequency, $\lambda = 30$ (i.e. $f = 96$ kHz). Note that the long axis of the vortex structures appears to point towards the “corners” where the bubble meets the wall.

$\epsilon \approx 0.03$). The azimuthal velocity is then scaled to the streaming velocity scale $U_1 = \epsilon U_0$ to yield $V_\epsilon$. We compare with theoretical calculations of the Lagrangian azimuthal velocity $V = -\partial_r \Psi$ for (i) velocities computed from experimentally measured $a_n$ and $\phi_{m,n}$ values (dashed line in Fig. 4.10(c)) and (ii) velocities computed without experimental input directly from the experimental values of $\lambda$ and $\gamma$ (solid line). Here, $\lambda$ and $\gamma$ are the dimensionless parameters: the driving frequency normalized by the frequency scale governing surface mode excitation ($\lambda$), and a viscous damping constant ($\gamma$), defined as $\lambda \equiv \omega (\rho a^3 / \Gamma)^{1/2}$ and $\gamma \equiv \lambda \delta^2 / 2$, where $\Gamma$ is the surface tension and $\rho$ is the density of the liquid [5]. The agreement between experiment and theory is very good, and we emphasize that neither the theoretical calculations nor the experimental streaming measurements involve any adjustable parameters. The radial distance of the vortex center (the zero of the curve in Fig. 4.10(c)) is also accurately reproduced, an important quantity for experimental design of vortex traps [134], size sorters [42, 43], or micromixers [5].

At higher frequency, the outward slip along the wall takes over as the dominant mechanism for streaming. In this wall-dominated high frequency regime, we find in both theory and experiment that the long axis of the vortex structures appears to point towards the
corners” where the bubble meets the wall, rather than towards the origin (see Fig. 4.11). In experiments, however, we find that the vortex centers are located closer to the wall than is predicted by the theory, which may be attributed to out-of-plane streaming flows driven by the walls parallel to the field of view confining the experimental set-up. The influence of these walls is expected to be significant in the large-\(\lambda\) (i.e. high \(f\)) limit, where wall streaming dominates.

4.5 Geometric effect on bubble dynamics and steady flow patterns

In the previous section, we have understood the frequency dependent bubble dynamics and streaming flow patterns, with one specific microfluidic set-up of \(w = 80\,\mu\text{m}, D = 1000\,\mu\text{m}\) and \(H = 1000\,\mu\text{m}\). In general cases, microfluidic set-ups utilizing microbubble streaming can have different dimensions. In this section, we investigate the effect of these geometric factors on the resonance characteristics of bubble dynamics and the resulting streaming flows.

4.5.1 Bubble with different shape

As we have mentioned in Chapter 2, we were able to control the bubble size by adjusting the temperature of the surrounding environment and the water chamber. Here we show that, for the same side channel width \((w)\), microbubbles of different protrusion length exhibit different resonance characteristics. We define a standoff distance \(\xi_a\) as the distance between the horizontal wall and the center of a circle that is fitted by the bubble outline (Fig. 4.12(a)). In Fig. 4.12(c) and (d), with larger \(\xi\) (i.e. a flatter bubble), the peaks of both \(\bar{a}_n\) and streaming intensity \(I_n\) are found at larger frequencies compared to bubbles with smaller \(\xi\).

Looking at Fig. 4.12(c) and (d), we also observe that the peak heights (i.e. absolute oscillation amplitude) are different. With a larger \(\xi\), the coupling between the volume mode
and surface mode is stronger. The surface mode amplitudes are larger, and thus result in stronger and more effective bubble streaming flows (larger $I_n$). This is in good agreement with experimental observations – stronger streaming flows occur with a flatter bubble interface. Detailed experimental measurement of the resonance frequencies of $\bar{a}_n$ in Fig. 4.13(a) indicates an increase of resonance frequency for flatter bubbles (i.e. larger $\xi$). The finding here is quite surprising, because a flatter bubble would have a larger radius by curve fitting the protruding segment, such that $a = \frac{w}{2\sqrt{1 - \xi^2}}$. Classical theory (Minnaert resonance) predicts an inverse relationship between bubble radius and volume resonant frequency, $f_0 = \frac{1}{2\pi a} \sqrt{\frac{3\lambda P_a}{\rho}}$ [153]. The contradiction suggests that other factors may be important for such bubble configuration, and are worthy of further investigation.

Because of the shift of resonance frequencies, the shape of the microbubble affects the frequency range to obtain specific streaming flow patterns. For a flatter microbubble, the bubble streaming flows due to higher order surface happens at a higher frequency. This is evident by comparing the streaming flow patterns for microbubbles driven at the same frequency at 26.7 kHz, in Fig.4.14. The streaming flow patterns clearly indicated the appearance of another pair of vortices near the bubble pole, in Fig.4.14(c); while the streaming flows are still only “fountain” loops for flatter microbubbles, in Fig.4.14(a)-(b). However, the streaming flow patterns are still governed by the streaming intensity curve.

In summary, by adjusting the degree of bubble protrusion from the side channels, we are able to control the resonance frequencies. A flatter bubble has higher resonance frequencies, and a larger bubble has lower resonance frequencies. This in turn suggests that the resonance is sensitive to bubble size. In order to maintain stable (reproducible) bubble streaming flow speed, a constant bubble size is thus required. While the resonance frequencies have shifted due to the size change, the general streaming flow patterns are consistent with the correlation discussed in the previous section 4.3. We can thus design the desired flow patterns (“fountain” or “anti-fountain”) when operating the bubble in the appropriate frequency range.
Figure 4.12: Protruding bubble of different shapes. (a) Standoff distance $\xi_a$ is measured between the horizontal wall and the center of a fitted circle of the bubble outline. (b1) to (b4) show four bubble shapes with different $\xi_a$. (c1) and (c2), (d1) and (d2) show the normalized amplitude $\bar{a}_n$ and the streaming intensity $I_n$ corresponding to the two extreme cases (b1) and (b4) respectively.
Figure 4.13: Resonance frequencies of $\tilde{a}_n$ for different bubble sizes ($\xi$) that protrude from the same side channel, ($f_1$, $f_2$, and $f_3$ are the resonant frequency of $\tilde{a}_1$, $\tilde{a}_2$, and $\tilde{a}_3$ respectively).

Figure 4.14: Streak images of microbubbles of different shapes (i.e. $\xi$) at the same driving frequency 26.7 kHz. Note that for the shape closer to semi-cylindrical shape, complex flow patterns due to higher order surface modes appear near the bubble pole.

4.5.2 Bubble of different aspect ratio

We have presented experimental evidence of the 2D characteristics of such cylindrical bubbles sandwiched between two plates. The comparison with a 2D theory also yields close agreement. In experiment, in addition to the regular channel depth $D = 100 \mu m$, we also fabricate shallower channels (i.e. smaller $D = 50 \mu m$) to examine the effect of the aspect ratio $D/w$. When comparing microbubbles of $D/w = 100/80 = 5/4$ with microbubbles of smaller $D/w = 50/80 = 5/8$, we observe almost identical positions of resonance frequencies,
but with different peak heights. The 2D asymptotic theory predicts resonance frequency position accurately, and better agreement in predicting the peak height with increasing $D/w$. This is because that a large $D/w$, the bubble approximates more closely to a semi-cylindrical shape. For smaller $D/w$, the amplitudes of the surface mode $\bar{a}_n$ as well as the streaming intensity $I_n$ are smaller, indicating more suppressed surface mode oscillations, due to the increasing damping of the sandwiched walls. This suggests that a high aspect ratio side microbubble is more effective in generating stronger streaming flows. This also means another advantage of using high aspect ratio microbubble – larger throughput or flow rate through the system, both because of increased channel opening and because of increased flow speed at equal power.

### 4.5.3 Bubble with different wall separation distance

When measuring the bubble resonance frequencies and studying the streaming flows, we have used a wide channel ($H = 1000 \mu m$), which is to minimize the effect of the other wall. However, in many practical applications such as particle manipulation, we want the bubble streaming flows to have a strong effect across the entire microfluidic channel, which then requires a narrow channel. Here we compare the bubble responses in two channels: one with $H = 1000 \mu m$, and another with $H = 250 \mu m$. From Fig. 4.16, we observe very similar bubble dynamics, in terms of both resonance frequencies, peak heights, and the resulting relative streaming strengths. This might be due to the relatively large channel separation, for even the narrow channel $H = 250 \mu m$, $H/a \approx 6$.

While the characteristics (resonance frequencies, $\bar{a}_n$, and $I_n$) of the bubble dynamics remain the same for both separation distances ($H = 1000 \mu m$ and $H = 250 \mu m$), the presence of the second wall influences the far-field streaming flow patterns, because the flows must have vanishing normal velocities towards the opposite wall. Fig. 4.17 shows the comparison of flow patterns between a wide and a narrow channels, where the driving frequencies are the same. Reducing the separation distance makes vortex loops more compressed and squashed.
In addition to the different shapes, as has been suggested by numerical calculation [154], the speed also increases with a smaller separation distance. Bubble streaming with small $H/a$ is thus a more effective means of transport and mixing – not only covering the whole channel region, but also with increasing flow speed. Also note that large separation $H$ leads to stronger 3D character compared to a narrow channel.
Figure 4.16: Effect of wall separation distance $H$ on normalized mode amplitudes $\bar{a}_n$ and relative streaming strength $I_n$: (a) and (b) for wall separation $H = 1000 \mu m$, and (c) and (d) for wall separation $H = 250 \mu m$.

### 4.5.4 Summary of geometric effects

From the above investigations, we see that the geometric aspects generally affect the different features of the flow, in different and largely independent ways. The bubble size influences the resonance frequencies – flatter bubbles have higher resonance frequencies $f_n$; the bubble aspect ratio $D/H$ affects the relative peak height $a_n$ – shallower bubbles have smaller surface mode amplitudes and thus weaker mixed mode streaming $I_n$; the wall separation distance mostly changes the appearance of the steady flow patterns – smaller separations cause more distorted and squashed streamlines. With these findings, we may thus manipulate certain
Figure 4.17: Steady streaming flow patterns with different wall separation distance $H$. (a1) and (a2) show the streaks at 16 kHz and 75 kHz inside a narrow microchannel ($H = 250 \mu m$). (b1) and (b2) show the streaks at 16 kHz and 75 kHz inside a wide microchannel ($H = 1000 \mu m$).

aspects of the flow by choosing the right geometric combination when designing fluidic devices.

4.6 Conclusions

The work in this chapter advances the fundamental understanding towards a quantitative description of microstreaming from a semi-cylindrical oscillating bubble attached to a wall, by experimentally measuring the oscillation modes of the bubble interface. The bubble dynamics characteristics are an indispensable input for the calculation of the mixed-mode streaming relevant in practical applications. It is shown that the relative amplitudes of surface modes to the volume mode provide a robust measure and predictive characteristic of the flow structure and that the features of the associated resonance structures in frequency space can be explained by asymptotic theory. As frequency increases and higher-order shape modes significantly contribute to the bubble oscillation, more intricate vortex structures de-
velop close to the bubble. The positions of the resonance peaks for the individual oscillation modes are well approximated by the expected resonance frequencies of free-bubble shape modes. However, the shape of the peaks (height and width) can only be understood through the coupling of different modes owing to the viscous effects in the boundary layers that govern the flow near the bubble interface and the wall. The relatively wide peaks help explain the robust flow field response obtained from bubbles in experiment, where an accurate fine-tuning of frequencies is generally unnecessary, and frequency drift does not compromise the flows. We have also varied several other parameters of the bubbles, such as bubble size, aspect ratio $D/w$, and wall separation distance $H$, to study how these affect the resonance frequency peaks and the relative streaming strengths. These geometric factors affect the bubble dynamics and streaming flows in a much independent way: bubble size determines the resonance frequencies – smaller protrusion has higher resonance frequencies; aspect ratio $D/w$ affects the higher mode amplitude – shallower channel has smaller higher mode amplitude; wall separation distance $H$ changes the overall flow portrait. From the practical perspectives, these findings are useful when certain aspects of microbubble streaming flows need to be tailored.
Chapter 5

Mixing strategies with microbubble streaming flows

In this chapter\(^1\), we present various designs of microbubble mixers utilizing steady streaming flows generated from acoustically driven microbubbles. Effective mixing strategies depend on both the temporal dynamics of the driving and the spatial organization of the flow. We have seen how we can understand the latter in Chapter 4, and the former is an application of time-dependent changes of such patterns. Accordingly, the strategies can be generally classified into two categories: (a) by modulating the acoustic driving pattern, such as the duty cycle and driving frequency, and (b) by controlling the arrangement of microbubble, such as the number, position, and orientation of the microbubbles. More specifically, modulating duty cycling will change the steady streaming flow directly by breaking steady flows into unsteady flows, and thus achieve more effective mixing. Modulating the driving frequency \(f\) can even alter the directions of the resulting far-field streaming flow. Strategically switching between drastically different flow patterns leads to improved mixing as well. On the other hand, when using multiple bubbles as actuating elements, the distance, position, and arrangement will affect the interaction between the streaming flows caused by individual bubbles. In particular, 3D flows can thus be achieved through proper arrangement and positioning of such microbubbles. Finally, we show that combining strategies of (a) and (b) can yield even better mixing for certain type of micro-mixers.

\(^1\)This chapter is adapted from part of Ref. [5, 155].
5.1 Introduction

5.1.1 Introduction to mixing and mixing measures

Mixing is a ubiquitous process that has many engineering and science applications, such as food and pharmaceutic industries, and chemical and polymer processes and reactions [156–158]. Mixing is the homogenization process of two or more solute species that have initially non-uniform concentration distribution within a system or domain. Truly homogeneous distribution of a solute (i.e. perfect mixing) is the homogenous state at the molecular level, which is achieved by the molecular diffusion of individual solute objects (e.g. molecules or nanoparticles). The diffusion process takes a time $\tau_D \sim l_s^2/D_m$ [159], where $D_m$ is the diffusion coefficient of the solute and $l_s$ is a characteristic gradient scale of the concentration field. For an initial distribution of white (solute) and black (no solute) schematically shown in Fig. 5.1(a), $l_s$ is also known as the striation thickness, which is defined as half of the typical thickness of the black and white striation period.

In general, mixing can be interpreted as consisting of two processes (or stages): stirring (or advection) and molecular diffusion [156–158,160]. Stirring refers to the advection of materials to reduce striation thickness (in Fig. 5.1(b)). Although mixing is the physical process involving stirring and diffusion simultaneously, molecular diffusion time scale will be much larger if the striation thickness is large enough. In other words, for solutes with low diffusion coefficient, mixing can be considered as two separate processes – first reducing the striation thickness through stirring, and subsequent diffusion over the individual striations (in Fig. 5.1(c)). It is clear that effective mixing is achieved through the reduction of striation thickness to the length scales that allow diffusion to smear out any concentration gradient in the desired time scale depending on the application requirement.

It is important to quantify how good the mixing process is. Several mixing measures have been described in the literature: such as (i) the standard deviation of the grayscale signal [45,161], $\sigma(c)$, as a fast and simple measure and (ii) the mixing variance [162], $\Phi^2(c-\bar{c})$, 

87
Figure 5.1: Schematic of a general mixing process. (a) initial distribution of white (solute, \( c = 1 \)) and black (no solute, \( c = 0 \)) in a domain. Striation thickness (\( l_s \)) is half of the white and black striation period. (b) reduced striation thickness through stirring (e.g. through chaotic advection, turbulence), with negligible diffusion. (c) molecule diffusion takes place and reduces the concentration gradients over striations.

as a more sophisticated measure. In the former, the standard deviation of the signal intensity within a region of interest (ROI) is calculated as,

\[
\sigma(c) = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (c_i - \bar{c})},
\]

(5.1)

where \( N \) is the total number of signal points evaluated, \( c_i \) is the signal intensity of each point, and \( \bar{c} \) is the intensity of the completely mixed liquid. All the above intensities are normalized, so that \( c = 1 \) for the initial bright liquid (containing solute), and \( c = 0 \) for the initial dark fluid (no solute). For a completely unmixed state (Fig. 5.1(a)), which contains bright liquid (i.e. \( c = 1 \)) in one half of the ROI and dark liquid (\( c = 0 \)) in the other half of the domain, the standard deviation is therefore \( \sigma(c)_{\text{unmixed}} = 0.5 \). And for a completely mixed liquid, the standard deviation of the signal intensity is \( \sigma(c)_{\text{mixed}} = 0 \). The value of \( \sigma(c) \) is often directly used as a measure of mixing quality. Several other alternative measures based on this concept are also commonly used, such as the coefficient of variation \( \frac{\sigma(c)}{\bar{c}} \) [163], and the mixing index that is defined as \( 1 - \frac{\sigma(c)}{\sigma(c)_{\text{unmixed}}} \) [146].

While the standard deviation is a simple and straightforward measure, it is more applicable
to flow systems where diffusion is the dominant mechanism of mixing. Take the concentration distributions in Fig. 5.1(a) and (b) as an example, based on the standard deviation method, $\sigma(c) = 0.5$ for both cases, while it is clear that the striation thickness in (b) is only half of that in (a). If mixing were to take place from these initial configurations through diffusion alone, the time required for (b) would be $1/4$ of that (a). In situations with complex striations but negligible diffusion, measures based on the standard deviation are not a proper indicator of the mixing state.

It is therefore recognized that length scale is an important consideration when quantifying mixing, particularly for cases where chaotic advection is dominant and molecular diffusion is negligible. Measures that take into account variation of the scalar field $c$ at all length scales (striation patterns of different length scales) are more appropriate [164]. Mixing variance is one of such multiscale mixing measures, which is defined as follows [162, 165],

$$\Phi^2(c - \bar{c}) = \sum_k \Lambda_k |c_k|^2, \quad \text{with} \quad \Lambda_k = (1 + 4\pi^2 k^2)^{-\frac{1}{2}},$$

(5.2)

where $c_k$ are the two-dimensional (2D) Fourier coefficients of the concentration field after subtracting the mean, i.e. $(c - \bar{c})$, such that $c - \bar{c} = \sum_k c_k e^{i2\pi(k \cdot x)}$. By the definition in Eq. (5.2), mixing variance is defined on a zero-mean concentration field. If we calculate the mixing variance of the concentration fields in Fig(a) and (b), we obtain $\Phi^2 = 0.0671$ and $\Phi^2 = 0.0339$ respectively. By this definition, we see that the mixing time due to diffusion is proportional to the square of the mixing variance, i.e. $t_m \propto (\Phi^2)^2$. The two-fold decrease in $\Phi^2$ indicates a decrease of the striation thickness, and reflects a better mixing state in Fig. 5.1(b) than that in Fig. 5.1(a).

### 5.1.2 Microscale mixing

The success of Lab-on-a-Chip/$\mu$TAS systems requires the devolvement, fabrication, and integration of many individual components and functions, such as pumping, sorting, heating,
mixing and various capabilities of sensing and detection [8,24,25]. Among these components, homogeneous mixing of chemical/biological samples and reagents is one of the essential preparation steps for biological and chemical reactions in µTAS systems. Some examples include polymerase chain reaction (PCR), large scale parallel chemical synthesis, genetic analysis, enzyme reactions, and bioreactors [166–170].

However, one challenge associated with micro-scale flows is the low Reynolds number and laminar flow characteristics. Taking a typical example, as shown in Fig. 5.4(a), in which the mean flow velocity is \( U = 2.67 \text{ mm/s} \), channel width is \( H = 250 \mu\text{m} \), and the Reynolds number is about \( Re = \frac{\rho U H}{\mu} = 0.39 \). At this Reynolds number, the two liquid streams form a stable interface between them. While low Reynolds number flows are useful for micro fuel cells [9], generating stable and precise chemical or nutrient gradient [171, 172], micro-patterning and fabrication [6], they are undesirable for achieving mixing. Without chaotic advection and turbulence, the only homogenization mechanism is molecular diffusion. With a typical diffusivity of biological molecules, \( D_m \approx 1 \times 10^{-10} \text{ m}^2\text{s}^{-1} \) the mixing time scale can be estimated as \( t_m \approx \frac{H^2}{4D_m} \approx 100 \text{ s} \). Residence time, \( t_r \), is the time during which the liquid travels through the device. This estimated diffusion time scale of 100 s is much longer than typical residence time of inch-sized LOC system, (assuming a reasonable channel length 1 cm, \( t_r = 2.75 \text{ s} \)).

The well known Taylor dispersion could be a mechanism of enhanced diffusion for pressure driven flows due to the parabolic velocity profile. The modified diffusion coefficient due to Taylor dispersion is \( D_{eff} = D_m(1 + \frac{1}{192}Pe^2) \) [133,173], where \( Pe \) is the Peclet number \( Pe = \frac{U H}{D_m} \). Using the same parameters above, \( D_{eff} \approx 2.3 \times 10^5 D_m \). However the effective diffusion coefficient is based on the assumption that the travelling distance \( L \gg \frac{H^2 U}{4D_m} = 0.17 \text{ m} \) [3,133,173], a condition that is not met by typical LOC devices. In consideration of the limited space and residence time available on the chip-sized platform, achieving fast mixing is therefore critical towards improving the overall efficiency of chemical and biological reactions.
Over the last two decades, there have been extensive studies on how to improve mixing in microfluidic environments. Some of the review papers offer excellent summaries of various strategies [18,174]. Generally speaking, these strategies can be classified into two broad categories: active and passive mixers. The active micro-mixers utilize various forms of external energy, such as pressure [175], electrokinetics [176], magnetism [177], acoustics [161], and thermal energy [56] to actively disturb and stir the liquids that need to be homogenized. Active micro-mixers often rely on more sophisticated parts (moving membranes/valves) [177] or micro-patterning (e.g. micro-electrodes [176]), and thus require more complex manufacturing processes. By contrast, passive micro-mixers take advantage of passive, non-moving parts to reduce the effective diffusion distance through introducing finer striations – alternating thin layers of solvent and solutes. Some other passive micro-mixers utilize twisted 3D structures to create chaotic advection (often as secondary flows) to expedite the mixing process [178]. One notable example is the well known herringbone mixer [163], in which secondary chaotic flow is induced with asymmetric grooves built into the channel wall.

Microbubble streaming flow is a powerful actuating mechanism, which rectifies macro scale acoustic energy into fast flows at small scale. The use of microbubble streaming flows for actuating purposes have become increasingly popular since the last decade. It has a number of advantages such as simple manufacture, parallel operations, and negligible heat generation. In the previous chapters, we have demonstrated and explored various manipulation of micro-sized objects with microbubble streaming flows. When used for mixing purposes, typical bubble microstreaming set-ups should favor mixing as well, because when superimposed on a directional flow, it forces fluid elements through a narrow gap between the bubble and vortex streamlines [42,43], reducing the diffusion distance between the two liquid streams in the process. Indeed, mixers based on microbubble steady streaming have been demonstrated in several set-ups by other researchers as well [39,40,67], with practical applications such as DNA hybridization [68]. However, most of these bubble-based micro-mixers employed a simple driving scheme (i.e. single frequency) and simple arrangement of bubbles (i.e. 2D
staggered bubbles). Although these microbubble mixers are conceptually simple and direct, are they effective?

In Chapter 4, we have understood the approximately 2D flow characteristics of bubble streaming flows due to the semi-cylindrical microbubbles. We further recognize that the 2D nature of the flow does not favor mixing. Strictly speaking, a 2D steady (time-independent) flow cannot be mixing at all by the Poincare-Bendixson theorem [179]. Here, our objectives are to propose methods or strategies to improve mixing with microbubble streaming. Specifically, we will study and compare various designs of acoustically driven microbubble mixers, with a focus on one of the most practically relevant mixers: a T-mixer with continuous throughput.

5.2 Experiment

5.2.1 Design and fabrication of micro-mixers

The T-shaped micro-mixers are fabricated in Polydimethylsiloxane (PDMS) by the soft-lithography technique that has been described in Chapter 2. We design a variety of micro-mixers, ranging from the very basic single bubble mixer (Fig. 5.2(b)) and two-dimensionally arranged multiple bubble (2D multi-bubble) mixers (Fig. 5.2(c)–(e)), to three dimensionally arranged bubble (3D multi-bubble) mixers (Fig. 5.2(f)(g)).

In the previous chapters, the bubbles are formed from blind side channels that are in the same plane (x-z) of the main channel. We will denote these bubbles as “side bubbles”, and they have semi-circular outlines when viewed in the x-z plane. Similar to side bubbles, we can also form bubbles that protrude from the top of the main channel in the x-y plane, and we denote these bubbles as “top bubbles”. Top bubbles have semi-circular outlines when viewed in the x-y plane. The 3D multi-bubble mixers integrate both side bubbles and top bubbles that are perpendicular to the main channel.

While the single bubble and 2D multi-bubble mixers are fabricated with a one-step lithog-
raphy process to create a planar master mold in SU-8; the 3D multi-bubble mixers require a two-step lithography process to achieve the 3D master mold (refer to sec. 2.2.3 for details). In addition, we vary parameters such as the separation distance between the bubbles, the order of arrangement, as well as the orientation of the top bubbles in 3D multi-bubble mixers to study how these parameters may affect mixing. Similar to the earlier experiments of particle manipulation, a piezoelectric transducer is glued to the bottom of the substrate to drive the microbubbles (Fig. 5.2(a)).

5.2.2 Equipment and materials

In the mixing experiments, a syringe pump is used to infuse two liquid streams through the two inlets: one liquid stream being a glycerol-water mixture (23% by wt) with fluorescent particles (radius $r_p = 50\, \text{nm}$, Life Technologies) and the other stream being density-matched but without fluorescent particles. We use a mercury-vapor lamp as the light source through an epi-fluorescence attachment. The excitation filter wavelength is about $460\, \text{nm}$ and the emission filter wavelength is about $520\, \text{nm}$. Under the illumination, the emission light from the fluorescent particles is captured by a high-speed camera. Videos are captured and saved for later analysis. At low fluorescence concentration, the gray scale intensity (fluorescent signal $c$) of the image is proportional to the fluorescein/fluorescent particle concentration in the liquid [180, 181]. In our experiment, the fluorescent nanoparticle solution has low concentration of $\approx 1\%$ by weight. Thus the fluorescent signal distribution is used to quantify mixing of the two liquid stream, as many other studies in the literature [146,180,182].

Throughout the experiments, we use the syringe pump to infuse liquids into the fluidic device. It is well known that syringe pumps produce unsteady flows even with extra care taken. According to the measurement by Korczyk et al. [183], the oscillation period of a syringe pump is estimated as $T = \frac{\alpha_t \pi d_{syr}^2}{4Q}$, where $\alpha_t$ is the pitch of the screw thread of the syringe pump, $d_{syr}$ is the syringe diameter, and $Q$ is the flow rate. In our experiments, with syringe diameter $d_{syr} = 5\, \text{mm}$, and flow rate $Q = 1\, \mu\text{L/min} = 0.06\, \text{mL/hr}$, we expect an
Figure 5.2: (a) Schematic of the experimental set-up of mixing. And example mixer designs: (b) single side bubble mixer, (c) multiple side bubble mixer, (d) multiple top bubble mixer, (e) slanted top bubble mixer, (f) 3D arranged side and top bubble mixer, and (g) 3D arranged side and slanted top bubble mixer.

oscillation period of about 20 minutes. However, we observe unsteadiness with a timescale of about 1 second. This much shorter unsteadiness may be due to the flexible plastic tubing used the experiment, unlike the metal tubing in the experiments of Korczyk et al. [183]. There might still be a large oscillation period ($\sim 20$ mins) predicted by [183]. In order to minimize the effect of unsteady flows due to the syringe pump, we choose carefully a time duration during which the flows are steady, and average the mixing measures from the images.
over this duration. For the cases of duty cycling and frequency switching, the effect of the unsteadiness is insignificant, because the duty cycling or frequency switching timescale $\tau$ is much smaller than the unsteadiness timescale ($\sim 1\text{ s}$).

### 5.2.3 Data analysis

In describing the fluorescent intensity, we normalize the gray scale intensity by the intensities of the two liquid streams before they meet at the junction (in Fig. 5.4). We select a region of interest (ROI) downstream of the microbubble, with a size of $L_x$ and $L_z$ (in Fig. 5.4(a)). The normalized signal intensity is defined as $c \equiv \frac{C - C_{\min}}{C_{\max} - C_{\min}}$, where $C_{\max}$ and $C_{\min}$ are the signal intensities of the bright and dark fluid respectively before they meet at the T-junction. Therefore, the initial conditions are: the liquid containing fluorescent particles has $c = 1$, and for the other liquid stream $c = 0$. By this definition, once the two liquids are completely mixed, $\bar{c} = 0.5$.

To compute the mixing variance, we first subtract the mean $\bar{c} = 0.5$ from the scalar field $c(x,z)$ within the ROI. We also normalize $x$ and $z$ by the dimensions of the ROI ($L_x$ and $L_z$), as shown in Fig. 5.4(a). Following Fourier decomposition, we then determine the Fourier coefficients corresponding to each wave vector and compute the mixing variance with Eq (5.2). Since the initial concentration distribution is similar to the one depicted in Fig. 5.1(a), we will normalize $\Phi^2(c - \bar{c})$ by the value of this initial un-mixed configuration ($\Phi_0^2 = 0.0671$), i.e. $\phi^2 = \Phi^2(c - \bar{c})/\Phi_0^2$, for the remaining sections in this chapter.

Note that the Stokes-Einstein diffusion coefficient of our nanoparticles is so small ($D_p = 2/3 \times k_B T / (6\pi \mu r_p) \approx 1.6 \times 10^{-12}\text{m}^2\text{s}^{-1}$) that the multiscale mixing variance can therefore be used to analyze the advection of the striations only. For such small diffusivity, and the short residence time in the set-up (typically a few seconds), the diffusion is negligible. This is clearly evident by the stretching-and-folding patterns of bright and dark fluid remaining visible throughout our field of view. The short residence time scale relative to the molecular diffusion time scale also allows us to evaluate and optimize the mixing effect of the flow field.
advection independent of diffusion effects. Note also that our flow is a practically relevant, continuous-throughput flow (rather than a flow in a confined space, where the fluid elements never leave the volume) – there is only finite residence time available for mixing. For our optical measurement system, we determine that the detection limit is about $\Phi_{\text{limit}}^2 \approx 10^{-4}$ (or $\phi_{\text{limit}}^2 \approx 0.0015$), by measuring the mixing variance of the completely homogeneous liquid.

Imaged at the middle plane of the fluidic channel, the fluorescent signals acquired from the 2D image can be interpreted as the depth averaged signals. Thus, the 2D measure $\Phi^2$ is appropriate for quantifying mixing if we restrict the measurement to a depth average sense. However, if there are truly 3D structures in the flows (which can be constructed as we will see later), neither $\sigma$ or $\Phi^2$ will be sufficient to describe the 3D spatial distribution of the fluorescent intensity. In this case, 3D fluorescein signal should be acquired with confocal microscope imaging in order to determine the 3D concentration distribution. Moreover, information of the 3D flow field which can be obtained with APTV (sec. 2.4.3), will be useful to compute other mixing measures, for example Lyapunov exponents [184], for the purposes of characterizing and comparing the performance of different mixer designs.

5.3 Results and discussion

5.3.1 Single bubble mixers

We first focus on a very basic type mixer known as T-mixer with a single microbubble protruding from a side channel, as shown in Fig. 5.4. We will start with a simple acoustic driving pattern (i.e. continuous and fixed frequency) to understand the flow and mixing characteristics resulting from it, and then will propose methods to improve the mixing efficiency.
5.3.1.1 Low frequency, continuous driving

As demonstrated in the earlier chapters, when superimposing a Poiseuille flow and a “fountain” bubble streaming flow due to low frequency driving, there exists a thin gap between the bubble interface and the upstream closed loop vortex (Fig. 5.3). When two liquid streams go through this thin gap $d_{\text{gap}}$, the striation thickness (i.e. diffusion distance) is thus effectively reduced to approximately $d_{\text{gap}}/2$ from the whole channel width $H/2$. As bubble streaming flows become stronger (i.e. smaller $s$), $d_{\text{gap}}$ decreases, such that $d_{\text{gap}} = sH$ in the limit of small $s$ [42, 43].

For complete homogenization to take place, we can estimate the required $d_{\text{gap}}$ as follows. The diffusion distance between the two initial liquid streams is $l_s^o = H/2$, and reduces to $l_s \approx d_{\text{gap}}/2$ when going through the thin gap. With a small enough $s$, $d_{\text{gap}} \approx sH$. The time required for complete mixing through diffusion is therefore $\tau_D \sim l_s^2/D_p = d_{\text{gap}}^2/(4D_p)$. In the meantime, the residence time through the gap is $\tau_g \sim a/u_s$, where $u_s \sim \epsilon^2a\omega$ is the streaming velocity scale, so we have $\tau_g \approx 1/(\epsilon^2\omega)$. If complete mixing is to happen, we have $\tau_D \leq \tau_g$, i.e.

$$d_{\text{gap}} \leq \sqrt{\frac{4D_p}{\epsilon^2\omega}}. \quad (5.3)$$

In the experiments, we quantify the mixing quality using the fluorescence signal $c$ from a window of size 250 $\mu$m $\times$ 250 $\mu$m that is centered at 575 $\mu$m downstream of the bubble and indicated by a square box in Fig. 5.4(a). This window is located at the furthest downstream position in the field of view. We choose it at the furthest possible location away from the bubble, so that the flow field resumes to the Poiseuille profile, and the fluorescence distribution represents a steady output due to the bubble streaming flows. We use both measures: (i) $\sigma(c)/\bar{c}$ and (ii) $\phi^2$, and smaller values indicate better mixing for both measures. As the driving voltage increases (Fig. 5.4(a) to (d)), we observe increasing size of the upstream vortex and thinner gap ($d_{\text{gap}} = sH \propto 1/\epsilon^2 \propto 1/V^2$ for small $s$). The corresponding mixing measures, $\sigma(c)/\bar{c}$ and $\phi^2$, are plotted in Fig. 5.4(e) and (f). Both measures show better
Figure 5.3: Schematic of two liquid streams flowing through the gap. (a) Bubble streaming flow superimposed with a Poiseuille flow creates one upstream and one downstream closed-loop vortices. Two liquids entering the main channel are funneled through the gap between the bubble surface and the upstream vortex. (b) Zoomed view near the bubble surface shows the reduced diffusion distance $\approx d_{\text{gap}}/2$ from the original channel width $H/2$.

mixing with increasing driving voltage. Furthermore, the two measures show similar trends – mixing starts to improve when streaming flows are strong enough to form a upstream vortex, consistent with the earlier streak visualization in Fig. 3.7.

In Fig. 5.5, we plot the modulus of the Fourier coefficients $c_k$ of different wave number $k$ or $(k_1, k_2)$, where $k_1$ and $k_2$ are in the $x$ and $z$ direction respectively. Here, smaller values of $k_1$ or $k_2$ represent larger striation thickness. The modulus, $|c_k|$, can be interpreted as the fluctuation amplitude of the intensity signal over the striation length scale of $H/(2\sqrt{k_1^2 + k_2^2})$, and in the direction of $(k_1, k_2)$. Note that appreciable fluctuation amplitudes $|c_k|$ occur in the $z$ direction only. And as the driving voltage increases in Fig. 5.5(a)–(d), we observe decreasing fluctuation amplitudes, which contribute to the decreasing mixing variance $\phi^2$ (i.e. better mixing), defined by Eq. (5.2).

However, even with the strongest streaming flow used in the experiment, there are still clearly striations of different gray scale intensities downstream. As we will see in the following estimation, this is due to the incomplete mixing in the thin gap between the bubble and the upstream closed vortex. With Eq. (5.3), diffusivity of the fluorescent particle, and typical experimental parameters $\epsilon = 0.1$, $f = 25\text{kHz}$, complete mixing will require a gap size
Figure 5.4: Mixing in microstreaming flows combining a steady channel flow (right to left) with bubble streaming at low driving frequency ($f = 27.1 \text{kHz}$) (a) bubble is not excited; (b) bubble is driven at Voltage = 40 V; (c) bubble is driven at Voltage = 70 V; (d) bubble is driven at Voltage = 90 V; (e) coefficient of variation of the grayscale signal, $\sigma(c)/\bar{c}$; (f) mixing variance, $\phi^2$, calculated based on the sampling window indicated as a square box in (a), at different driving voltage. For both measures used here, smaller values indicate better mixing. Also note that the scale is log in (f), but linear in (e).

$d_{\text{max}} \approx 0.08 \mu \text{m}$, which is far smaller than the gap size we can reliably establish, about $1 - 2 \mu \text{m}$ [42, 43]. If the solute to be mixed is a typical molecular solution, such that $D_m \sim 1 \times 10^{-10} \text{m}^2\text{s}^{-1}$, this will in turn require a gap size of $d_{\text{max}} \approx 0.5 \mu \text{m}$, still smaller than the size we can normally obtain. The stretching mechanism through the gap certainly improves mixing, but yet complete mixing requires a very small $d_{\text{gap}}$. Although such small gap size can be achieved through reducing $\bar{u}_p$, this will compromise the throughput because of the smaller flow rate.
Figure 5.5: The modulus of the Fourier coefficients $c_k$ of the first 24 wave vectors. The flow condition is at low driving frequency ($f = 27.1$ kHz): (a) bubble is not excited; (b) bubble is driven at Voltage = 40 V; (c) bubble is driven at Voltage = 70 V; (d) bubble is driven at Voltage = 90 V.

5.3.1.2 Single frequency, modulating duty cycle

To address the inefficiency of the simple continuous driving strategy, we now consider the effect of breaking up the steady flow through low-frequency duty cycling: ultrasound of fixed amplitude and frequency is turned on and off alternately for fixed time intervals $\tau \gg 1/f$. As seen in Fig. 5.6(e), this strategy does improve mixing, if $\tau$ is large enough (note the case...
of continuous driving is plotted at $\tau = 0$). Even with only 50% of the energy consumption of the continuous driving, the mixing quality is nevertheless improved. Duty cycling is advantageous because, during the “off” part of the cycle, new unmixed fluid enters the region that – during the “on” part of the cycle – is taken up by the streaming vortices, quickly stretching out this fluid through the gap into thin bands. This mixed region is then advected downstream when the driving is turned off again, leading to bright and dark fluid regions distributed across the entire channel height $0 \leq z \leq H$.

We can also understand this better mixing from another perspective: for the continuous driving case, the two liquid streams are only accessible to the thin gap, while for the “on” and “off” modulation, the liquid streams are accessible to both vortices, due to the Poiseuille convection during the “off” duration, which means more effective stirring by both vortices in addition to the thin gap mixing mechanism. The duty cycling modulation reduces the mixing variance measure in particular, which emphasizes uniformity on large length scales [162]. With a continuous driving scheme as in (Fig. 5.4), this process cannot happen. Furthermore, the unsteadiness of the modulation breaks the Poincare-Bendixson theorem, and irregular (chaotic) trajectories become possible.

Maximum efficiency is expected when the time scale $\tau$ allows transport of unmixed fluid across an entire bubble diameter (i.e. a scale of $w$). If $\tau$ is much smaller, the liquid distribution does not change much during the intervals of no driving. In Fig. 5.6(b), we observe small wrinkles due to the duty cycling, but still observable striation patterns in the $z$ direction. However, if $\tau$ is much larger, unmixed fluid flows by without being mixed and this will cause non-uniform signal intensity over large length scales in the $x$ direction, thus resulting in poor mixing again. In Fig. 5.7, we plot the modulus of the Fourier coefficients $c_k$ for different duty cycle intervals. Initially, the increasing interval $\tau$ effectively reduces the fluctuation amplitude at large striation length scales (Fig. 5.7(a)-(c)) and improves the mixing quality. However, a large $\tau$ causes a non-zero $c_{(0,0)}$ Fourier coefficient (i.e. the mean is not 0.5), and results in poor mixing.
Figure 5.6: Mixing in microstreaming flows combining a steady channel flow (right to left) with duty cycling modulated bubble streaming at low driving frequency ($f = 27.1$ kHz) (a) duty cycle interval $\tau = 5$ ms; (b) duty cycle interval $\tau = 20$ ms; (c) duty cycle interval $\tau = 50$ ms; (d) duty cycle interval $\tau = 200$ ms; (e) mixing variance, $\phi^2$, for different duty cycle intervals.

In Fig. 5.8, we plot the mean signal intensity $\bar{c}$ of the entire sampling window for different $\tau$. The fluctuation of $\bar{c}$ as a function of time can be interpreted as the fluctuation of signal intensity in the $x$ direction, because the distance advected by the Poiseuille flow, $x_p$, is $x_p = \bar{u}_p \tau$. For one complete duty cycle (on for $\tau$ and off for $\tau$), the distance is therefore $x_\tau = 2\bar{u}_p \tau$. For smaller $\tau$, the fluctuation $\bar{c}$ has both smaller amplitude and smaller period ($2\tau$), as shown in Fig. 5.8(a)–(d). For larger $\tau$, the fluctuation of $\bar{c}$ increases both in amplitude and over a longer period, (see Fig. 5.8(e)(f)). The fluctuation period is proportional to the fluctuation length scale, $x_\tau$.

Among the intervals that are explored, we observe best mixing at $\tau = 50$ ms, which has the smallest mixing variance computed from both the $x$ and $z$ directions. In our experiments, with $w = 80 \mu$m and $\bar{u}_p \approx 1.3$ mm/s, we find $\tau_p \approx 60$ ms, in close agreement with the best
Figure 5.7: The modulus of the Fourier coefficients $c_k$ of the first 24 wave vectors. The flow condition is at low driving frequency ($f = 27.1$ kHz): (a) duty cycle interval $\tau = 5$ ms; (b) duty cycle interval $\tau = 20$ ms; (c) duty cycle interval $\tau = 50$ ms; (d) duty cycle interval $\tau = 200$ ms.

mixing values ($\tau = 50$ ms) in Fig. 5.6(e). The mixing variance, in particular, detects this minimum very consistently at different driving amplitudes.
5.3.1.3 Modulating driving frequency

However, mixing can be further improved by alternating streaming between lower and higher frequencies. We again adopt time intervals of length $\tau$, but now we switch between a low frequency ($f_l = 27.1$ kHz) and a high frequency ($f_h = 91.3$ kHz) driving at voltages resulting in comparable bubble oscillation amplitudes. The (still ongoing) Poiseuille advection part of the cycle is then further aided by a different bubble streaming advection, which also redistributes the fluid, but does so on a shorter time scale, as the flow speed of streaming is faster than the Poiseuille flow speed. As seen in Fig. 5.9(a)–(d), the short time scales...
lead to a finer structure of stretching-and-folding stripes in the flow, together with the desired large-scale mixing across the z-direction of the channel. The mixing quality is thus significantly enhanced using this method, decreasing $\phi^2$ from 0.12 to 0.08 (Fig. 5.9(e)). The finer striations of the fluid are desirable as the time scale of eventual diffusive mixing is governed by their length scale.

Note that this approach only works when the switch frequencies belong to different modes of oscillation and thus substantially different flow fields. With the analysis of frequency dependent bubble interfacial oscillations and streaming flow patterns in Chapter 4, such a mixing strategy can be designed in advance. In our example, we have chosen $f_l = 27.1 \times 10^3$ Hz and $f_h = 91.3 \times 10^3$ Hz, as the flow fields display complete reversal of orientation, aside from other quantitative differences. These two driving frequencies thus maximize the difference in flow fields between the cycles, as well as result in improved mixing quality.

Figure 5.9: Mixing in microstreaming flows combining a steady channel flow (right to left) with frequency modulated bubble streaming at low driving frequency ($f_l = 27.1 \times 10^3$ Hz) and high frequency ($f_h = 91.3 \times 10^3$ Hz) for different switching intervals. (a) $\tau = 5$ ms; (b) $\tau = 20$ ms; (c) $\tau = 50$ ms; (d) $\tau = 200$ ms; (e) mixing variance, $\phi^2$, at different switching interval.
The mixing measure in Fig. 5.9(e) for frequency switching indicate better mixing throughout, and additionally show a second minimum at a much smaller time scale $\tau_f$, while the first minimum is the same as the case of low frequency driving alone, $\tau_p = 60 \text{ ms}$. By the same arguments as above, we estimate this second minimum as $\tau_f = w/u_s$, where the characteristic velocity of streaming \cite{42} replaces that of the Poiseuille velocity. The streaming velocity near the bubble surface in our experiments is $u_s \approx 10 \text{ mm/s}$, resulting in $\tau_f \approx 8 \text{ ms}$, again in close agreement with the location of this second minimum. Apart from the obvious advantage of mixing on smaller time scales, the more accurate mixing variance criterion also rates this minimum at $\tau_f$ as better in mixing quality. At these two minimums that give similar mixing variance $\phi^2$, we observe different visual appearances of the fluorescence signal field: there are smaller striation patterns in the $x$ direction but still larger striations in the $z$ direction for the case of $\tau = 5 \text{ ms}$; while there are larger striations in the $x$ direction but smaller striations in the $z$ direction. Note that the estimates of $\tau_p$ and $\tau_f$ rely on effective averaging over the oscillatory flow during a modulation time interval. As $\tau_f \gg 1/f$ even for the shortest $\tau$ employed in our experiments, this ensures the proper time scale separation between the steady streaming flows and modulated unsteady flows.

5.3.2 Multi-bubble mixers

Through the above studies with a single bubble micro-mixer, we have gained much insight on how to make it more efficient. Another strategy is to take advantage of parallel driving of multiple microbubbles. Indeed, this can be done simply by creating more blind channels to form multiple microbubbles. Next we will explore these mixer designs in details.

5.3.2.1 2D multi-bubble mixer

The first multi-bubble microbubble mixer is a direct extension of the single bubble mixer by placing more blind side channels alternately along both sides of the main channel of the T-mixer. Following the previous arguments, we can estimate the residence time through
the thin gaps as \( \tau_g \sim Na/u_s \approx N/(\epsilon^2\omega) \), where \( N \) is the number of bubbles. Accordingly, by equating the residence time \( \tau_g \) and the diffusion time scale, the gap required is thus \( d_{\text{gap}} \ll \sqrt{N \frac{4Dp}{\epsilon^2\omega}} \). This estimation is based on the assumption that the distances between the bubbles are far enough and the streaming flows of the bubbles do not interact with each other. Thus, this is a rather conservative estimation by just adding up the gap durations through each bubble. With four bubbles and for solute with diffusivity \( D_m \sim 1 \times 10^{-10} \text{m}^2\text{s}^{-1} \), the gap size required is about \( 1 \mu\text{m} \). With \( N = 16 \), the gap size required is then \( 2 \mu\text{m} \), which is the size that can be achieved reliably. For sufficient large driving voltage to form an upstream vortex, in Fig. 5.10(b)–(d), visually we see that the mixing becomes better after each microbubble. However, due to the small spacing between bubbles, we evaluate the mixing variance only at a window located the end of the field of view. Also note that, with strong enough streaming flow (Fig. 5.10(d)), the interaction between the streaming flows of neighboring bubbles seems to cause unsteadiness (see the slightly non-smooth appearance of the striations). Due to this unsteadiness, liquids can now enter the upstream or downstream vortex loops, further improving mixing due to the advection in the vortex.

In addition to placing bubbles on the side, we can also create top bubbles by making blind channels on top of the main channel (see Fig.5.2(a) and sec. 5.2.1). This is achieved with a two-layer lithography process in making the SU-8 mold. Fig. 5.11(a1)–(a4) shows the a multiple top bubble micro-mixer at different driving voltage. Due to the 2D flow characteristics as understood earlier, such placement of microbubbles causes two-dimensional steady streaming flow motions in the \( x-y \) plane. Because the initial distribution (or variation) of solute (fluorescence) is along the \( y \) direction only, with half of the channel occupied by the fluorescent solution, the 2D flow motions in \( x-y \) plane cannot redistribute solute in the \( y \) direction. Consequently, a microbubble mixer of this type is not effective in mixing enhancement, evident from the persistent bright and dark striations as well as the marginal improvement of mixing variance in Fig. 5.11(a4).

Also note that some of the mixing occurs due to what we may term the “end effect” of
Figure 5.10: A multiple side bubble micro-mixer driven continuously at low driving frequency ($f_i = 27.1$ kHz): (a) Voltage = 50 V; (b) Voltage = 60 V; (c) Voltage = 70 V; (d) Voltage = 80 V; (e) mixing variance, $\phi^2$, at different driving voltage.

a microbubble, where there often exists a small gap between a top bubble and the main channel. In Fig. 5.12(a), the microphotograph shows two small gaps between the top blind channel and the main channel. For the fluidic device used in the experiment, the top blind channel has a length smaller than the main channel width. When a top bubble forms from such a top blind channel, it has a rounded end near the main channel instead of forming full immobile contact onto the main channel. Bubble streaming flows from imperfect top bubbles can induce complex 3D fluid motions near the end (Fig. 5.12(b)). The small gaps are caused by the micro-fabrication process. Even though the photo-masks have alignment marks, some misalignment is inevitable due to the resolution of the mask aligner. Moreover, inconsistent exposures of the photo-resist may also cause slightly larger (overexposure) or smaller (underexposure) features than designed. As a side note for future fabrication, if a top bubble without gaps is desired, the top blind channel can be designed to be longer than the main channel width. This will ensure that the top blind channel establishes proper
(a1) 

(a2) 

(a3) 

(a4) 

(b1) 

(b2) 

(b3) 

(b4) 

Figure 5.11: Multiple top bubble micro-mixer driven continuously at low driving frequency ($f_l = 28.9 \text{ kHz}$): (a1) Voltage = 50 V; (a2) Voltage = 70 V; (a3) Voltage = 90 V; (a4) mixing variance, $\phi^2$ for parallel top microbubbles. (b1)–(b4) are for the multi slanted top microbubble mixer.

Although these parallel top bubbles are not very effective in enhancing mixing directly, one additional degree of freedom is that we can make slanted bubbles, as shown in Fig. 5.11(b1)–(b4). By placing top bubbles at an angle, the bubble streaming flows are now in planes different from the $x$-$y$ plane. The slanted microbubbles thus cause material exchange in the $z$ direction. With the same number of bubbles and the same driving amplitude and frequency, we observe a better mixing from the slanted top bubble-mixer ($\phi^2 = 0.17$) than
Figure 5.12: Top view of a top microbubble. (a) optical microphotograph indicates gaps between the top bubble and the main channel wall, and the resulting round ends of the bubble. (b) trajectories of two incoming particles: a particle near the channel wall exhibits a complex 3D trajectory; while a particle coming along the center of the main channel stays in the \( x-y \) plane.

the parallel top bubble mixer \((\phi^2 = 0.45)\).

### 3.3.2.2 3D multi-bubble mixer

As have been demonstrated by other various micro-mixer designs [18,174], 3D chaotic flows are preferred over 2D flows in order to stretch and fold the fluid elements and to reduce the diffusion distance. With the 2D flows produced in the \( x-y \) and \( x-z \) planes by side and top bubbles, we can now easily construct 3D flows by superposition. Fig. 5.2(a) shows schematically a T-mixer with one side bubble and one top bubble. Note that side and top bubbles are essentially the same – having the same bubble dynamics and steady streaming flow characteristics – except that the orientations are different. Since both side and top bubbles are perpendicular to the main channel, the interaction of the flows can give rise to mutually transverse vortices, 3D streamlines that span across the entire main channel. Consequently, such 3D convoluted flows will lead to better mixing.
In Fig. 5.13, we compare the mixing performance of three micro-mixers: a 2D multi-bubble mixer, a 3D multi-bubble mixer made of units containing one pair of side bubbles and one top bubble, and a 3D multi-bubble mixer made of units containing one side bubble and one top bubble. The mixing variance is calculated at 7 different locations along the main channel, as indicated by rectangular boxes in Fig. 5.13(a1). Each of the sampling windows is of size $240 \mu m \times 120 \mu m$. We also vary the driving voltage to the piezoelectric transducer.
From Fig. 5.13(b1)–(b3), we observe consistent better mixing with 3D multi-bubble mixers compared to 2D mixer, especially at larger driving amplitude. At the end of the set-up and for high driving voltage, both of the two 3D microbubble mixers have reached close to the homogeneously mixed state ($\phi^2_{\text{limit}} \approx 0.0015$, in sec. 5.2.3). Among the two versions of 3D mixer, although they show comparable magnitude of mixing variance, the design with one pair of side bubbles and one top bubble is better. In particular, the rate of decay of mixing variance $\phi^2$ with respect to the traveling distance is faster, indicating a more effective mixer design.

5.3.3 Combined strategies

In the earlier sections, we have presented two general strategies to effectively use microbubble streaming flows for mixing: one controlling the acoustic driving patterns (i.e. duty cycling and frequency switching), and the other utilizing multiple bubble interactions. Can a com-
combination of them lead to even better mixing? Here, we demonstrate such combined strategy with one 2D and one 3D multi-bubble micro-mixers. Like the earlier experiments with the single bubble mixer, we employ both duty cycling and frequency switching techniques to them. Fig. 5.14 shows the mixing variance at 7 different locations, for the cases of duty cycling (a) and switching frequency (b) respectively. The time interval of duty cycling and switching is \( \tau \), and \( \tau = 0 \) is for continuous low frequency driving. Fig. 5.14 suggests that when combining with a 2D multi-bubble mixer, both duty cycling and frequency switching can yield better mixing, with a suitable interval \( \tau \) chosen – both around 50 ms. In Fig. 5.14, by comparing the two methods with a continuous low frequency driving case (with \( \phi^2 = 0.05 \)), we also observe that frequency switching works better than the duty cycling, by achieving \( \phi^2 = 0.02 \) and \( \phi^2 = 0.03 \) respectively at the downstream of the mixers.

![Figure 5.14: Combined strategy of mixing with a 3D bubble micro-mixer: (a) with duty cycling of different interval \( \tau \) and (b) with switching between low \( f \) and high \( f \) of different interval \( \tau \).](image)

In Fig. 5.15, we determine the mixing variance at 7 different locations of a 3D multi-bubble mixer, for the cases of duty cycling and switching frequency respectively. Fig. 5.15(a) shows that for all different \( \tau \) studied, duty cycling is not improving the mixing with a 3D multi-
bubble mixer. From the experiments with single bubble mixer, duty cycling decrease the non-uniformity in the $z$ direction by breaking steady 2D flows. Here, owing to the interaction of the side and top bubbles, the flow in the main channel is 3D. This means that duty cycling is not necessary, and reduction of microbubble streaming flows here actually results in less effective 3D flows and thus less mixing. On the hand, with the frequency switching, mixing is further improved with a proper choice of $\tau$ in Fig. 5.15(b). Mixing with $\tau = 50$ ms is better than both continuous low frequency driving as well as other switching intervals. The flows are more complicated because of the combined 3D and unsteady nature. Numerical simulations would be necessary to gain a better understanding. Measurement of 3D velocity fields with APTV (see sec. 2.4.3) will provide useful information about the flows. The 3D velocity data will also allow quantifiable characterization of the mixing efficiency, for example by computing Lyapunov exponents.

### 5.4 Conclusions

In this chapter, we have investigated various strategies and microfluidic device designs to study the mixing performance with microbubble streaming flows. These microfluidic devices with rectangular cross section shapes are the most commonly encountered type in practical applications – resulting from lithography based microfabrication. When microbubbles are formed between two sandwiching plates, microbubble streaming flows exhibit 2D character, which limits the mixing if such microbubbles are simply driven in a continuous fashion. This is because although superposition of the “fountain” bubble streaming with the Poiseuille flow produces a thin gap which reduces the diffusion distance, the condition to ensure complete mixing relying solely on thin gap mechanism requires very small gap size ($\sim 0.5 \mu$m) and is often difficult to achieve.

To circumvent such an inefficiency, we have proposed two general strategies: modulating acoustic driving, and utilizing different arrangements of multiple bubbles. Starting with
single bubble mixers, we study the flow and mixing that is modulated by acoustic driving (i.e. duty cycling and frequency switching). Duty cycling breaks the steady flows into unsteady flows and causes smaller striation thickness across the entire channel, leading to better mixing. In a similar fashion, frequency switching establishes unsteady periodic flows of two completely different flow orientations (“fountain” and “anti-fountain”), and the alternating far-field streaming flows redistribute the liquid in a more efficient way for subsequent mixing. With the mixing variance $\phi^2$ as a measure, duty cycling decreases $\phi^2$ by a factor of 2, and frequency switching decreases $\phi^2$ by a factor of 3, when compared to the case of low frequency continuous driving. Although the flows (sec. 4.5) and thus the mixing characters are dependent on the geometric parameters of the set-ups, we demonstrate significant improvement of mixing by these strategies.

We have also explored different designs of multiple bubble mixers: two dimensionally arranged side bubbles and three dimensionally arranged side and top bubbles. The 3D arrangement of side and top bubbles introduces interaction of 2D streaming flows in different planes (i.e. $x$-$z$ and $x$-$y$) and results in 3D steady flows to expedite the mixing process.

These strategies presented in this chapter also highlight the flexibility of microbubble streaming based micro-mixers. When the microbubbles are duty cycling modulated or frequency modulated, the steady microbubble streaming flows are converted to unsteady flows – a character of active mixers. When driven continuously at a fixed frequency, these bubbles establish steady flows – resembling passive mixers. This flexibility represents a unique feature of microbubble streaming flows. Finally, combining these two strategies can result in even better mixing when proper times of modulation are chosen.
Chapter 6

Conclusions and future work

6.1 Summary

Throughout this dissertation, we have advanced the understanding of microbubble streaming flows from both practical and fundamental perspectives. Experimentally, we have demonstrated a way of producing powerful steady streaming flows with acoustically driven microbubbles. These microbubbles are formed as both an integrated and compatible part of the existing microfluidic technologies, which are fabricated using the technique of soft lithography. Such an integration has a number of advantages – larger oscillation amplitude and faster flow speed, simple manufacturing, negligible heat generation, and parallel operation, when compared to other actuating mechanisms.

When these bubbles are not excited, they act merely as part of passive boundary of the microfluidic channels. However, when excited with acoustical pressure, they are capable of generating strong local flows to shape the flow topology inside the entire main channel, i.e. through a superposition of bubble streaming flow and Poiseuille flow. We further show the usefulness of such combined flow fields for various particle manipulations – switching, sorting, focusing, and enriching. These manipulations rely on a unique mechanism for selective trapping by size: unlike any other microfluidic approaches, microbubble streaming flows achieve this size selectivity and integrated sorting by introducing virtual gaps into the flow and thus allow for sorting at the position of largest speed in the flow. We also propose a quantitative criterion of the size selection, which is confirmed with experimental measurement. By simply adjusting the relative strength of the streaming flows, the size selection
can be tuned. Using microbubbles as basic actuating elements and exploiting the trapping and releasing mechanisms, we demonstrate the versatility of microbubble streaming flows: affecting both spatial and temporal concentrations of the particles. This general concept for virtual confinement and flow partitioning without introducing any moveable elements is applicable to many other types of micro-scale flow configurations as well.

From a fundamental perspective, the second part of our work has bridged a gap between engineering applications of microbubble streaming flows and the underlying physical principles. In this part, we have focused on a quantitative understanding of the frequency dependent flow patterns. By varying the driving frequency, we have observed a variety of steady flows, ranging from “fountain” flows to completely reversal flow – “anti-fountain” flows. Through experimental measurement of different mode amplitude and phase, we quantify the frequency dependent bubble dynamics and thus establish a correlation between the bubble dynamics and steady streaming flow patterns. We find that at low driving frequency, the “fountain” flows are the result of mixed mode streaming – interaction of different bubble oscillating modes; while at high driving frequency, the “anti-fountain” flows are due to the dominant wall streaming. Such an understanding allows flexible tuning of the flow patterns to suit different applications.

Equipped with a better understanding of the flow characteristics, we turn our attention to applications – specifically the design of more efficient micro-mixers that are based on microbubble streaming flows. Most of the existing bubble-based mixers use a fixed frequency and continuous driving to generate steady flow for mixing enhancement. Although simple and direct, this implementation is efficient. We proposed and demonstrated two general strategies for more effective mixing utilizing microbubble streaming flows: controlling the acoustic driving pattern and constructing 3D arranged microbubbles. We also show the flexibility of operating our microbubble mixers either as active mixers or as a 3D steady passive mixer. We manufactured various types of bubble micro-mixers and measured the mixing performance by applying both our novel techniques (duty cycle and frequency modulation,
6.2 Ongoing and future work

The current investigation has built a solid foundation of bubble streaming flows and opens up new possibilities of future studies. We envision a number of interesting aspects worthy of further exploration. Some of the work is already ongoing (but not described in details in this thesis).

6.2.1 Continuous filtering

Our earlier technique relied on trapping microparticles into the upstream vortex (closed streamlines) and subsequently releasing them to achieve switching, focusing and sorting. We now propose a concept of a continuous size filtering device utilizing bubble streaming flows. As schematically shown in Fig. 6.1(a), the basic continuous filtering fluidic device consists of one main channel (A–B) and two side channels (C–D). In the main channel, a Poiseuille flow from inlet A to outlet B is driven by a syringe pump past a bubble in a side channel as before. Opposite the bubble there are two more side channels connected to a second syringe pump in such a way that fluid is pushed into the main channel from inlet C and withdrawn from the main channel towards outlet D, with equal flow rate $Q_2$, which also ensures flow conservation through the main channel. This flow configuration leads to a separatrix forming between the A–B and C–D flows even without bubble streaming. The important and unique feature of bubble streaming flow is the fast velocity ($\approx u_s$) within the gap that ensures the narrowness of the gap. If a solid bump or protrusion is used instead of
the bubble, the no-slip boundary condition on the solid bump will have a zero velocity on the bump surface and results in a larger gap (i.e., particles of smaller size cannot be sorted or separated).

Figure 6.1: Concept of a continuous size filtering device with two inlets and two outlets: (a) two syringe pumps inject and withdraw liquid simultaneously with flow rate $Q_1$ between A and B and $Q_2$ between C and D. The streaming flows from an ultrasound driven bubble modulate the separatrix between the A–B and C–D flows. The gap between the bubble interface and the separatrix sets the critical size whether a particle is filtered. Particles with radius larger than the gap are kicked across the separatrix and transported to D. Smaller particles continue to flow towards B. The streak visualization of the separatrix are shown when the bubble is not excited (b) and when bubble is driven by the ultrasound (c). Switching of microparticles ($a_p = 5 \mu m$) to two different outlet ports are demonstrated in (d) and (e). In the experiment, the flow rate ratio between the two flows is $Q_2/Q_1 = 10$. 
Introducing particles of various sizes at \( \mathbf{A} \), particles of radius \( a_p > d_{\text{gap}} \) collide with the bubble boundary layer and are kicked across the separatrix into the \( \mathbf{C} - \mathbf{D} \) flow. But unlike for the case of closed streamlines, they are now simply transported away and collected at \( \mathbf{D} \). Streak visualizations of the flow field indeed confirm the feasibility of tuning the gap size with microbubble streaming flows (in Fig. 6.1(b),(c)). When the bubble is not excited, the bubble interface merely serves as a stress-free slip boundary, and forms a gap between itself and the separatrix (in Fig. 6.1(b)); when the bubble streaming flow is activated, the flow velocity near the bubble interface increases and thus decreases the gap size (in Fig. 6.1(c)). In the experiment with microparticles, the switching works as what we expected. When the bubble is not excited, the microparticles are transported to \( \mathbf{B} \) (in Fig. 6.1(d)). And upon activation of the bubble streaming flows, all of the particles are directed to the other outlet \( \mathbf{D} \), (in Fig. 6.1(e)).

Compared to the trapping and releasing mechanism, the new continuous filtering method has several advantages. First, it is now possible to have a much larger throughput. With the trapping and releasing mechanism, an upstream closed-loop vortex is required in order to create the gap structure for trapping particles. The gap size is estimated to be \( d_{\text{gap}} = sH = \bar{u}_p/u_sH \) in the small-\( s \) limit. For a typical channel width of 250 \( \mu \text{m} \), a gap size of 5 \( \mu \text{m} \) requires \( s = u_p/u_s = 0.02 \). For a typical microbubble streaming velocity \( u_s \sim 30\text{mm/s} \), the Poiseuille velocity \( \bar{u}_p \) should not exceed 600 \( \mu \text{m/s} \). However, the new concept does not need to form a closed vortex, as the flow through the side channel \( \mathbf{C} \) and \( \mathbf{D} \) sets up a closed loop by itself. The gap size, \( d_{\text{gap}} \), between the bubble interface and the separatrix is set by both the flow rates ratio \( Q_2/Q_1 \) and the bubble streaming strength \( s \). An increase of \( Q_2/Q_1 \) pushes the separatrix towards the bubble interface. Additionally, increased velocity \( u_s \) further reduces the gap size. In Fig. 6.1(e), we switch the microparticles to outlet \( \mathbf{D} \) successfully, with a total flow rate more than 10 times higher than before. Secondly, with the new concept, no accumulation or particle-particle collision occurs, and thus there is no escaping of the large particles and re-mixing with the small particles. Moreover, the basic
principle demonstrated in Fig. 6.1 can be applied iteratively to achieve size fractionation of a continuous distribution of particle sizes (i.e., for polydisperse suspensions). In Fig. 6.2(a), two devices in series are depicted with higher $Q'_2$ and smaller $s$ in the downstream device, which therefore has a smaller gap width. In this fashion, several size ranges can be separated and filtered out from the same channel flow.

Practical microfluidic devices employing this general concept have many potential biomedical applications, such as filtering biological cells by stiffness, and filtering macromolecules. In many contexts of biological interest, the deformability (mechanical stiffness) of cells carries a wealth of information about malignancy (cancer diagnostics [185,186]), cell age or disease e.g. in red blood cells [187,188], or degree and type of differentiation in stem cells [189]. For a device depicted in Fig. 6.2(b), there exist strong velocity gradients ($u_s \propto 1/r^n$ with $n > 2$, see sec. 3.3 and sec. 4.4.2) near the bubble interface, which can exert strong enough shear stresses to deform or even lyse biological objects [35,38]. When a mixture of biological objects of similar size but different stiffness is transported through the thin gap, we envision a separation due to the difference in mechanical deformability: a softer biological object deforms more, and squeezes through the gap, being transported to $B$; while a harder object deforms less, crosses the separatrix, and is transported to $D$ (Fig. 6.2(b)). In order to study deformable objects, giant unilamellar and multilamellar phospholipid vesicles are ideal candidates to start with, because phospholipid vesicles are a commonly used model system for cell studies, and vesicles of different stiffness can be formed through electroformation [190]. We have produced vesicles with the electroformation technique and introduced them into the bubble streaming flows (Fig. 6.2(d)(e)) [191], and thus providing the necessary preliminary data for future systematic investigation of this topic.

Long-chained macromolecules, notably polymers and DNA, are of enormous importance to the biosciences as well as the chemical processing industry. And because of intrinsically small quantities, these molecules have to be manipulated on the microscale [192,193]. Macromolecules of long enough backbone are flexible (on the length scale of a persistence
Figure 6.2: (a) Schematic of multiple connected devices for iterative size fractionation. By controlling the flow rate through the side channels, $Q_2$ and $Q'_2$, two different gap sizes are set, enabling filtering particles of different size in a continuous manner. (b) Principle of filtering by stiffness or deformability of the transported particles. A mixture of stiff (dark gray) and soft (light gray) biological objects of equal size are introduced. The latter deform sufficiently in the elongational shear flow in the gap to squeeze through to $B$, while the former cross the separatrix and are collected at $D$. (c) Coils of macromolecules (e.g. $\lambda$-DNA) are introduced at $A$. The relaxation time of one molecular species (red, spherical) is too small to undergo a coil-stretch transition and crosses the separatrix, while that of the other (magenta, elongated) results in a Weissenberg number above critical. (d) Giant unilamellar and multilamellar lipid vesicles grown by electroformation. (e) Trajectories of vesicles in the streaming flow of a single bubble (preliminary data).
length [194]) and generally assume a coiled state in the absence of external forces. In the case of long DNA strands, such as those from λ or T4 phages [193,195], these roughly globular coils have diameters of up to a few µm. This would place them in the right size range for the filtering applications considered here, but the sensitivity with respect to backbone length would not be very good. However, in flows with strong enough elongational components, such molecules undergo a coil-stretch transition as first recognized by de Gennes [196]: Once the Weissenberg number \( W_i \), the product of (dominant) molecular relaxation time \( \tau \) and shear rate \( \dot{\gamma} \), is sufficiently large (typically \( W_i > 10 \) [197,198]), the coil unravels into a stretched molecule aligned with the direction of elongation. Such a stretched molecule easily slips through a very narrow gap if the direction of elongation is also the flow direction (Fig. 6.2(c)). The flow field induced near the bubble fulfills all these conditions: The shear rate is high (easily \( \dot{\gamma} \gtrsim 10^{−100} \text{s}^{-1} \) [35–37] and contains elongational shear components [38]. Together with typical relaxation times \( \tau \) of seconds for λ-DNA in aqueous solution [197,199], the flow will induce a coil-stretch transition at sufficiently high shear (i.e., sufficiently low \( s \)). At this point, \( s \) becomes an even more sensitive means of control: as the relaxation time depends on the molecular size and structure, even subtle changes in DNA length can be decisive for whether the molecule stays coiled (and will be filtered out of the main flow) or stretches and is collected at the main outlet [198,200].

### 6.2.2 Advanced control of bubble streaming flows

Throughout this work, we have gained fundamental understanding on the bubble dynamics, and the steady streaming flows by studying a sessile semi-cylindrical bubble attached onto a solid wall boundary. The wall boundary plays an important role, in affecting both the bubble dynamics and the resulting steady streaming flow patterns, through the coupling of boundary layers of wall streaming flow and bubble mixed-mode streaming flow. With standard micro-fabrication techniques, it is fairly easy to manufacture microfluidic devices that have different topography. We have manufactured fluidic devices that have complex...
shapes, such as placement of a solid bump, or slanted wall boundary. One simple illustration is to make an angled/slanted wall neighboring to the side channel where a microbubble forms, as shown in Fig. 6.3. The angled boundary walls significantly suppress the “anti-fountain” loops up to high frequencies of about 100kHz and beyond. This effect becomes more prominent with more acute angle of the slant (Fig. 6.3).

Figure 6.3: Microbubble streaming flow patterns with angled walls at different driving frequency. In the top row, the walls have an angle of 15°: (a) 34.8 kHz, (b) 80.8 kHz, (c) 102.8 kHz (d) 141.8 kHz. In the bottom row, the walls have an angle of 30°: (e) 34.8 kHz, (f) 80.8 kHz, (g) 102.8 kHz (h) 141.8 kHz.

Fountain loops of the bubble streaming flows are especially useful for various manipulations of microparticles, and for improving mixing. Recall that the streaming velocity $u_s \sim \epsilon^2 a(2\pi f)$, so that driving the bubble at a higher frequency increases the transport speed of the liquid as well as the throughput. With microbubbles attached to a straight wall, the flow reversal to the “anti-fountain” loops happens at a lower frequency. For bubble size $a \approx 40 \mu m$, the transition frequency is about 50 kHz (see sec. 4.3.1 and Fig. 4.2). As shown in Fig. 6.4(a), at $f = 116.3$ kHz, the superposition of a Poiseuille flow and the “anti-fountain” streaming flow does not form the thin gap structure, so that the sorting and switching does not happen. However, if an angled wall is used instead, at $f = 116.3$ kHz the streaming flows still have the “fountain” orientation. This enables particle manipulation based on the trapping and releasing mechanism (Fig. 6.4(b)).

Additionally, introducing asymmetric structures near the bubble breaks the symmetry of bubble streaming flow (a similar effect was previously shown with a 3D bubble [36]), which
may result in novel transport phenomena. Here we demonstrate one such device: a solid bump of semi-cylinder shape is placed near to the blind channel from which a semi-cylindrical bubble forms. The whole fluidic device is a closed network (Fig. 6.5(a)). When driven by the acoustic pressure from a piezoelectric transducer, the microbubbles establish steady streaming flows. In this case, the driving frequency is about 25.4 kHz, and the flows have the “fountain” orientation. The solid bump prevents vortices forming, and breaks the symmetry. This bubble-bump pair introduces a net flow inside the fluidic network (Fig. 6.5(b2)), which is shown by the particle streaks from an imaging window on the opposite side (in Fig. 6.5(c)). This concept adds another useful function – liquid transport/pumping to the toolbox of the microbubble streaming based microfluidics.

In order to understand the most fundamental principles about bubble streaming flows, our study has been focused on the single bubble or multiple bubbles of the same size. It
is worthwhile to integrate bubbles of different sizes and/or to drive them at two or more different frequencies. These will result in complex flows, but are also likely to have additional functions and benefits.

### 6.3 Closing remarks

Fundamental studies of microbubble streaming flows are instrumental towards the effective use of the intriguing fluid-mechanical phenomenon of streaming as an actuating mechanism for liquid transport at small length scales, with particularly useful applications in microfluidic and lab-on-a-chip systems. In this work, we have made progress on various aspects on
this topic, including the development of several experimental techniques to characterize the bubble dynamics and streaming flows, a first-time fundamental understanding of the unique frequency dependence of microbubble streaming flows, as well as the demonstration of practical applications of particle manipulation and liquid mixing. This dissertation work will benefit researchers of many fields including fluid mechanics, bioengineering, biophysics, and micro/nano-fabrication. The general concept of versatile and tunable particle manipulation by streaming is practically applicable in a broader context for sorting and purification of biological cells and large molecules, and for the characterization of cell mechanical properties.
Appendix A

Liquid droplet on micropatterned substrate: imaging contact line and friction force measurement

In this chapter\(^1\), we describe experimental work on the characterization of solid-liquid interactions on micro-patterned surfaces: measuring both the contact line of liquid droplets and the friction force of moving liquid droplets. We develop a novel experimental technique to quantitatively study the interaction forces as a function of the geometry and defect characteristics of the micropatterns.

We have seen that deformability and contact line dynamics and position (see bubbles of different $\xi$ in sec 4.5.1) are important for flow phenomena. Here we have a closer look at the relation between interface shapes and contact line shapes, and pay special attention to contact line dynamics that is strongly influenced by the presence of pinning sites. And such problems have garnered considerable attention recently because of the importance of controlled static and dynamic wetting.

A.1 Introduction

Controlled wetting properties have many important industrial applications, such as self-cleaning, anti-wetting, heat transfer enhancement by drop-wise condensation, and improved film coating quality with controlled roughness [202–206]. With the advancement of micro/nano-fabrication techniques, researchers are now able to modify the wetting properties by engineering various micro/nano-textured surfaces, many of which are inspired by examples in nature, such as the well known “lotus effect” [203]. Substrates with micropatterns, partic-

\(^{1}\)In collaboration with Huan Li and Jimmy Hsia [201].
The contact angle is a commonly used macroscopic parameter to characterize the wetting property between a surface and a liquid. The contact angle is defined as the angle at which the liquid/air interface meets the solid surface on the contact line (CL), shown in Fig. A.1(a). The smaller the contact angle, the easier does the liquid spread over the surface.

The two classic models relating surface roughness to wetting properties are the well known Wenzel [207] and Cassie-Baxter [208] model (Fig. A.1(b)(c)). These two models predict the contact angle under the assumption that the system is in thermodynamical equilibrium. For a droplet in the Wenzel state, the droplet wets the entirety of the rough surface covered by the droplet outline. The contact angle $\theta_W$ is given by

$$\cos \theta_W = r \cos \theta_Y, \quad (A.1)$$

where $r$ is the roughness of the solid, defined as the total surface area divided by the projected area. For a droplet on a solid surface with an air pocket trapped underneath, the contact angle $\theta_{CB}$ is given by the classic Cassie-Baxter model [208],

$$\cos \theta_{CB} = \phi (1 + \cos \theta_Y) - 1, \quad (A.2)$$

where $\phi$ is the area fraction of solid with respect to contact interface, $\theta_Y$ is the intrinsic
contact angle or Young’s angle of the solid.

However, for micropatterned surfaces, contact angle is often not an adequate measure of wetting properties for a number of reasons. First, the measured contact angle is not the actual contact angle [209]. Due to the variations in the local roughness, the surface of the droplet is distorted near the contact plane. While well defined for a perfectly smooth surface, the measured contact angle on micropatterned surface is only a global average value (known as apparent contact angle), which is often fitted from the droplet shape.

Second, the apparent contact angle is not a unique value. The contact line is typically pinned locally by chemical inhomogeneities or roughness of the material, thus preventing the apparent contact angle from reaching the value compatible with the lowest Gibbs free energy. This phenomenon is called contact angle hysteresis [210]. Thus, each measured contact angle corresponds to a metastable energy state, and is not a uniquely determined value.

Thirdly, the Cassie-Baxter model is derived on the assumption of thermodynamic equilibrium, without considering the three-phase contact line topology. There have been speculations that the contact angle depends on more details than just the area fraction of the solid. Some works [211] claim that the more distorted the contact line, the more hydrophobic the surface; while others [212] suggest that a smooth, continuous CL leads to more hydrophobicity.

Lastly, apart from the contact angle, more information is needed in determining whether the droplet is in Cassie mode or Wenzel mode. Although the transition from Cassie mode to Wenzel mode has been extensively studied [213–215], a technique for direct observation of the contact area during such transitions is still not available.

In addition to the apparent contact angle, the contact area size and the shape of static droplet on micropatterned surface are important to understand the wetting characteristics from a microscopic level. To further quantify the dynamics of wetting between a moving droplet and micropatterned substrates, particularly the dynamics aspects – contact line
pinning, de-pinning and distortion, and the friction force experienced by the moving drop, direct visualization of contact line motion and precise force measurement are curial, but not readily available. In this part of the work, we present novel experimental techniques for simultaneous quantitative measurements of droplet shape and contact-line pinning forces, both with a spatial resolution at the single-defect level and capable of fast time resolution. With high-speed imaging and sensitive force sensors, forces and deformations of droplets and substrates in relative motion will be determined simultaneously.

A.2 Experiment setup

A.2.1 Fluorescence imaging of droplet contact line

We use fluorescent dye (fluorescein disodium C_{20}H_{10}Na_{2}O_{5}, molecular weight of 376.27 g/mol, Fisher Scientific) for imaging the contact line. A small amount of fluorescein (6.44 ppm, parts per million by weight) is mixed into DI water, which is the liquid used for all experiments. The small amount of fluorescein leaves the properties of the DI water practi-
Figure A.3: Fluorescence images of the contact area for a 20 µL droplet on top of PDMS cylindrical micropillars with a diameter of 57.1 µm and center-to-center spacing 138.4 µm. Pillars are in a square lattice. (a) droplet is in the Cassie-Baxter state; (b) droplet is in the Wenzel state.

cally unchanged, in particular the surface tension. The contact angle of pure DI water on a smooth PDMS surface is measured to be 114°, the same as that of the fluorescein-DI water.

The experimental setup is schematically shown in Fig. A.2. A PDMS (Polydimethylsiloxane) substrate, which may have various surface micropatterns, is placed on a microscope stage. A fluorescein-DI water droplet with volume of around 10 µL is gently dispensed with a pipette onto the substrate. The illumination is from a mercury lamp. A digital camera (Phantom V310) captures the bottom view images/videos of the contact line through an inverted microscope (Olympus IX71), which has an excitation filter of 460 nm, and an emission filter of 521 nm.

A typical image taken by the microscope is shown in Fig. A.3(a). The focal plane is set at the pillar-top-surface. In Fig. A.3(a), the outermost circumference is the brightest. Light emitted by the fluorescein from the equator of the droplet has almost no refraction, because it does not travel through the curved droplet surface. The light intensity is thus the highest

132
at the droplet circumference in the image. Between the circumference and central area, there is a slightly brighter annulus. It indicates that the water does not contact with sample surface. The light from the inside of the droplet travels through the surface which curves out of the surface of pillar top. It then gets refracted, and leads to such an annulus. The central dark area indicates the air pocket trapped between pillars. Pillars with top surfaces contacting the droplet appear brighter than their surroundings and thus can be identified in the image. The contact line, which encloses the bright pillars, can be outlined. Finally, the shape of the CL and the size of the contact area can be determined quantitatively.

### A.2.2 Friction force measurement

The experimental setup for force measurement is schematically shown in Fig. A.4. In addition to the fluorescence imaging capability, this setup includes a microforce sensor and a data acquisition system, which enable measuring and recording of the force data.

The microforce sensor and data acquisition system used in the experiment are purchased from Nanoscience Instruments, Inc. The sensor, FT-S540, with force measurement range of ±180 μN, is developed by FemtoTools. It is a capacitive MEMS micro force sensor that has a high sensitivity (90 μN/V), good linearity (< 4%) and fine resolution (0.3 μN at 1000Hz). The sensor includes internal circuit which converts the measured load into the output voltage (0-5 V). The force signal is then recovered from the measured voltage using the data acquisition system (micro controller, FemtoTools), and the output is recorded on a computer using Labview software.

As shown in Fig. A.5, the sensor probe is made of silicon, with a width of 300 μm. The tip is even smaller, 50 μm in width. A sphere of PDMS ~ 2 mm in diameter is glued to the probe in order to contact the droplet and prevent detachment while the droplet is dragged on the target surface. A PDMS sphere is used because it has a large enough surface area to allow the droplet to adhere. PDMS is a hydrophobic surface, so it is not fully wetted by the droplet. It can largely maintain the droplet shape and protect the sensor from being wetted.
Figure A.4: Schematic of the apparatus used to measure the friction force of a liquid drop sliding on a micropatterned surface. In the experiment, the drop adheres to the PDMS sphere by wetting, which is then connected to a force sensor. The microscope stage, controlled by computer, is moving away from the sensor at a speed of $V$. The drop will then slide on the surface while remaining in contact with the stationary PDMS sphere. The friction force is measured by the force sensor and collected with a data acquisition system. The motion of the CL and contact area are captured by a high speed camera (Phantom v310, Vision Research) using a fluorescence microscope.

Figure A.5: Close up image of the force sensor tip, on which a PDMS sphere is glued. (a) top view, and (b) side view.
A.3 Contact line of static and moving liquid droplets

Here we present some representative results of the contact line visualization of static as well as moving droplets, while detailed and systematic measurements with different liquid drop volume, and on a variety of micropatterned substrates are found in Ref. [201].

A.3.1 Static droplet

For a static drop on micropatterned PDMS substrate, we present some typical results and findings from a micropatterned surface with circular pillars with square lattice arrangement. The pillars have a height of 13-25 µm and a diameter of $D = 26 - 59$ µm in square lattice. The area fraction $\phi$, defined as the ratio between pillar area and the total surface area, ranges from 0.1 to 0.7 by variation of the center-to-center spacing. The droplets are observed by the fluorescence microscope, and the images of the contact area are recorded. The size and shape of the contact area are then acquired and analyzed.

Fig. A.6(a) and (b) show the contact area images of 7 µL droplets wetting substrates of $\sim 27$ µm diameter cylindrical pillars in square lattices with area fractions of 0.20 and 0.59. Fig. A.6(c) and (d) show the contact area images of 7 µL droplets on $\sim 43.7$ µm diameter cylindrical pillars in square lattices with the area fractions of 0.13 and 0.38. Fig. A.7(a)-(d) show the contact area shape variation with different substrates described in Fig. A.6(a)-(d) for larger droplets with the volume of 20 µL.

For small droplet of 7 µL, as shown in Fig. A.6(a)-(d), the CL shape is closer to an octagon, especially for substrates with large pillars. Four sides of the octagon, at the top, bottom, left and right, coincide with the square lattice direction (10). Also, between every two adjacent lines mentioned above, the CL also adopts a “shortcut” to minimize the total free energy, and thus forms other four line segments along the lattice direction (11). Additional line segments along the lattice directions (12) connect the square vertices built by the lines in directions of (10) and (11) to form the closed octagon shape.
Figure A.6: Contact area of a 7 µL droplet wetting micropatterned surfaces with micropillars in square lattices.

If the droplet volume is increased from 7 µL to 20 µL, shown in Fig. A.7(a) to (b), all contact areas tend to approach a circular shape. As indicated by Marmur et al. [216, 217], as the droplet volume is increased, the CL approaches a circular shape on a large scale. The droplet will appear almost as a spherical cap. The contact angle will approach the value predicted by Cassie equation, because if the size of the droplet becomes sufficiently large, the effect of local roughness on the droplet advancing or receding is negligible. The droplet is thus more free to reach its minimum energy state, which results in a circular contact area.
Figure A.7: Contact area of a 20 \( \mu \)L droplet wetting micropatterned surfaces with micropillars in square lattices.

### A.3.2 Moving droplets

Our experimental setup is capable of direct observation of the dynamics of the moving contact lines. Here we show one example: the contact line evolution during the coalescence of two droplets. Because of the high-speed (1000 - 3000 fps) capability, we are able to study the dynamical aspects on a fast-time scale.

In this experiment, two 7 \( \mu \)L drops of fluorescein-DI water mixture are deposited gently using a micropipette on a micropatterned surface consisting of PDMS micropillars. The
surface is hydrophobic and the contact angle is greater than 90°. Two drops are placed close to each other, but not touching. The distance between two drops is determined by the closest points on the curved surfaces of the drops above the contact area, rather than the nearest distance between contact lines. A syringe is used to push air streams to move the drop gently towards the other without changing the shape of the contact line. The drop surface will deform and then touch the other drop. The coalescence happens in milliseconds. The fast coalescence is captured at 3100 frames per second by our high speed camera (Phantom v310, Vision Research) through an inverted microscope (IX71, Olympus) from the bottom.

Fig. A.8 shows the coalescence process. At $t = 0$ ms in Fig. A.8(a), the drop at the top of the figure is blown toward the other droplet, and the liquid bridge starts to form between the drop surfaces. For both drops, the contact line is not yet in motion. Due to the negative surface curvature in the bottleneck region of the liquid bridge [218], the surface tension will drive the composite drop surface of this region to expand along its center line. However, this region still does not touch the substrate. As shown in Fig. A.8(b), the CL of two drops has not changed. As the neck region continues to expand along the center line, at 2.26 ms in Fig. A.8(c), the liquid bridge reaches the substrate and the CL forms a dumbbell shape. Fig. A.8(d) and (e) show that the CL continues to expand in the center region, forming nearly straight lines. The CL at the top and bottom of Fig. A.8(e) has not yet begun to recede. In Fig. A.8(e)-(h), the contact line shrinks at the top and bottom of the figure and migrates towards the center line and continues to expand along the center line.

In Fig. A.8(i)-(l), because of the surface energy and remaining kinetic energy, the newly formed long axis of the composite drop decreases in length and the short axis increases. The composite drop recovers its initial shapes, then continues to elongate and shrink in this manner with decreasing amplitude. Finally, because of the viscous dissipation, the composite drop will reach its equilibrium shape with nearly spherical surface and circular contact area. Low hysteresis of the hydrophobic surface to the CL motion, leads to a faster coalescence, but also a longer relaxation time for the composite drop to reach its final equilibrium shape.
Figure A.8: CL motion of two 7 µL droplets coalescing on the micropatterned surface with pillar diameter 43.7 µm and φ = 0.16.
Figure A.9: Measured force vs. displacement of the droplet as the droplet is dragged on a micropatterned surface. The surface consists of square pillars of side length 57.1 µm in a square lattice with center-to-center spacing of 134.8 µm. The initial slope of the force curve, $k \approx 102.9 \text{ mN/m}$, can be interpreted as the linear elasticity of a deforming droplet. In the inset, $k_s \approx 58.9 \text{ mN/m}$ is the elasticity and $W \approx 0.187 \times 10^{-9} \text{ J}$ is the work required by moving the droplet over the spacing distance between the pillars.

A.4 Friction force of moving liquid droplet

In this section, we present direct measurements of the friction force of a moving droplet on micropatterned surfaces. One unique capability of our experimental setup is the simultaneous observation of contact line motion and measurement of the force. With this technique, we establish a point-to-point correlation between the dynamics of the moving contact line and the force curve. A typical force vs. time curve is shown in Fig. A.9. The curve is obtained using an 11 points, un-weighed moving average. The micropatterned surface used in the measurement consists of square pillars of side length 57.1 µm in a square lattice with center-to-center spacing of 134.8 µm.

Snapshots of the corresponding bottom view, the contact line and contact area, captured by the microscope with a high speed camera are shown in Fig. A.10. The side view is monitored by another camera, the snapshots from which are shown in Fig. A.11.
Figure A.10: Bottom view of a 10 µL droplet being dragged to slide on a PDMS substrate, composed of square pillars of side length 57.1 µm in a square lattice with center-to-center spacing of 134.8 µm. (a) PDMS sphere on the sensor probe just touching the droplet; (b) maximum force generated during sliding. The contact area between the droplet and substrate is at the maximum. The lateral sides of the CL (top and bottom in the figure) will shrink towards the center; (c) steady state sliding of the droplet. The force generated during sliding maintains an almost fixed amplitude and period due to regular pinning and depinning of the CL at the trailing edge. The shape of the contact area remains unchanged with successive jumps of the CL at the trailing edge and migration of the CL at the leading edge.
Figure A.11: Side view of a 10 µL droplet being dragged on a PDMS substrate, composed of square pillars of side length 57.1 µm in a square lattice with center-to-center spacing of 134.8 µm. (a) PDMS sphere on the sensor probe just touching the droplet; (b) maximum deformation of the droplet, corresponding to the maximum force generated during sliding; (c) steady state sliding of the droplet. The force generated during sliding remains almost constant. The shape of the droplet remains unchanged, with successive detachment and attachment at the droplet’s trailing edge, and continuous wetting at the advancing edge.

The force evolution of a droplet sliding on a micropatterned surface can be divided into the following stages.

**Attachment:** When the sensor is brought into contact with the droplet, the droplet partially wets the PDMS sphere surface and adheres to it, shown in the side view Fig. A.11(a). The droplet is slightly displaced and a traction force is generated on the sensor upon adhering. The magnitude of the force depends on the relative location of the droplet and PDMS sphere. As shown at the beginning of the curve in Fig. A.9, the initial force is 29 µN in this trial.
At this moment, the contact area and contact line maintain their original shape, because of contact angle hysteresis (CAH), as shown in Fig. A.10(a).

**Force Increases:** As the microscope stage starts to move at a speed of 0.062 mm/s away from the sensor, the adherence between the PDMS sphere on the sensor tip and the droplet is strong enough so that the PDMS sphere holds the droplet in place as the micropatterned surface moves beneath it. The traction force needed to overcome the friction is thus measured by the force sensor. As shown in Fig. A.9, as the stage begins to move, the force increases monotonically before reaching a nearly maximum value. Initially, the force increase is almost in a linear fashion. It is similar to a uniaxial tensile test where the material response is linearly elastic before the upper yield point is reached. The contact area does not change during this process; the contact line at the front and back side of the contact area is pinned and does not move.

As the droplet is dragged further by the sensor, the force curve reaches the end of the linear region, and the force curve starts to oscillate. This happens because the contact line detaches periodically from the pillar tops at the trailing edge, and the droplet starts to move forward (if one imagines that the stage is stationary and the relative motion of the droplet is moving forward). In the meantime, the contact line at the front edge also begins to protrude forward. As the above phenomena are happening, the contact line also starts detaching at the two lateral sides (top and bottom of the contact area in Fig. A.10(a)). The detachment starts from the corners of the back and lateral sides and propagates forward. The contact line at the lateral sides thus breaks and shrinks towards the center of the droplet. Just before the contact line detaches from the last pillar at the outermost lateral sides, the total resistance force reaches a maximum.

It is seen in Fig. A.9 that near the start of the force curve, the force linearly increases with displacement of the substrate. The loading is very slow 0.062 mm/s, so it can be treated as a quasi-static process. The initial slope of the force curve, \( k \), can be interpreted as the linear elasticity of a deforming droplet.
**Maximum Friction Force:** The maximum force on the curve is the maximum resistant force generated during the dragging, denoted by $F_m$. The contact area takes a shell-shape, shown in Fig. A.10(b). It can be seen from the side view that the droplet deforms most at this moment, shown in Fig. A.11(b). Passing this point, the lateral sides of the CL continue to detach from the substrate and shrink towards the droplet center. Consequently, the force will decrease.

**Force Decreases:** The CL segments at the two lateral sides start to detach from the pillar tops, one after another, from the back side corners to the front side. As the CL detaches from the last pillar at the outermost lateral side, it jumps toward the center line, whose direction is parallel to the movement of the stage. During this process, the CL at the trailing edge continues to detach, leading to the periodic oscillation of the descending force curve in Fig. A.9.

**Steady state:** As two lateral sides of the CL jump toward the center line, the friction force decreases until the steady state is reached. Meanwhile, the trailing edge of the CL continues to detach and the leading edge continues to protrude, both in a periodic manner. When it reaches a steady state, as shown in Fig. A.10(c), the CL shape now is almost rectangular with rounded front and back ends and straight lateral sides. This CL shape in the steady state is due to the arrangement of the pillars, which is a square lattice, since the moving direction is along the $\langle 10 \rangle$ direction of the lattice, the CL tends to take short-cut across the nearest pillars in the lattice in order to reduce the total free energy. Thus, the CL forms a straight line at both lateral sides of the contact area. The front and back side are close to circular arcs.

The shape of the force curve during the steady state remains almost unchanged, jumping up-and-down around an average value, as shown in the inset of Fig. A.9. This average force value is denoted by $F_s$. The periodic sudden drop of the force curve is mainly caused by the periodic detachment of the CL from the pillar tops at the trailing edge [219, 220], especially the CL depinning from the last column of micropillars. At the advancing edge, the meniscus
continuously and smoothly lies down on pillar tops, which is essentially a wetting process. The resisting force caused by this wetting process can be neglected. During the steady state, after the CL detaches from the previous row of pillars, the dragging force increases linearly with displacement, as shown in the inset of Fig. A.9. The CL detaches again after the slide travels approximately the pillar center-to-center distance, jumping to the neighboring row. This cycle continues as long as the droplet remains on the substrate. The slope of the force-displacement curve, i.e., the stiffness of the CL, $k_s$, within each cycle is approximately a constant $\approx 58.9 \text{ mN/m}$ in Fig. A.9.

For the current sample, micropillars with side length of 57.1 $\mu$m in a square lattice with center-to-center spacing of 134.8 $\mu$m, the average amplitude of the force jump during the steady state is $4.6 \pm 0.6 \mu N$, shown in the inset of Fig. A.9. The amplitude is calculated by averaging all the maximum-to-minimum differences on the curve during the steady state. This is the magnitude of the force needed for the CL to make one set of detachment a column of pillars. As the force drops and the CL depins from the last column of pillars, for this sample, there are usually 3 – 4 pillars wetted in the last column. Thus, if one assumes that each pillar in the last column contributes equally, on average, the depinning from one pillar requires $\sim 1.2 – 1.5 \mu N$. The frequency of the force drop depends on both the translational speed and the spacing of the micropillars. In this trial, the translational speed is 0.062 mm/s and pillar center-to-center spacing is 134.8 $\mu$m. The period of each force drop is 2.17 s (or 0.135 mm if distance is used), which can be seen in the inset of Fig. A.9.

The video from the side view, one frame of which is shown in Fig. A.11(c), also shows that during the steady state, the droplet motion on the micropatterned surface is discrete and stepwise, microscopically, especially at the trailing edge. The deformation of the droplet is alleviated in the steady state, compared with the shape in Fig. A.11(b), in which the droplet is most deformed.

The maximum force $F_m$ and/or the steady state force $F_s$ is an indicator of surface hydrophobicity. If the surface needs larger force in order to move a droplet, it is sticky and
less hydrophobic. If the force is smaller, then a small perturbation, such as air blow acting on the droplet, will readily remove the droplet. In other words, the surface can efficiently remove water and thus it is more hydrophobic.

A.5 Conclusions

In studying microbubble streaming flows, we have developed and applied various experimental techniques (such as high-speed microphotography, fluorescence imaging, and imaging process) to quantitatively characterize the bubble dynamics as well the flow fields. Here, we show that these general experimental techniques can be applied to study deformable liquid interfaces in a different context – the wetting dynamics of liquid droplets on micropatterned surfaces. We (together with Huan Li and Professor Jimmy Hsia) have accomplished building a novel experimental platform for interface science and engineering that allow simultaneous force measurement and contact line imaging of liquid-solid interactions, both with a spatial resolution at the single-defect level and capable of fast time resolution. The experimental platform provides a quantitative testbed for long-standing theories of interfacial processes such as contact line pinning/de-pinning under different geometric and chemical conditions, thus benefitting many other fields, particularly surface chemistry and micro/nano-fabrication.
References


