Fachtagung "Experimentelle Strömungsmechanik"
5.–7. September 2023, München

Charakterisierung des Strömungsverhaltens in überlagertem Poiseuille-Fluss und Ultraschall-betätigtem Mikroblasen-Mikrostreaming mittels µPTV zur Einzelteilchensortierung

Characterization of Flow Behavior in Superimposed Poiseuille Flow and Ultrasound-Actuated Microbubble Microstreaming using µPTV for Single-Particle Sorting

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Mikroblasenströmung, Mikrofluidik, Sortierung von Einzelteilchen, Akustikfluidik
Microbubble streaming, Microfluidics, Single-particle sorting, Acoustofluidics

Abstract

One promising approach involves combining microbubble streaming and Poiseuille flow in lab-on-a-chip devices to perform particle manipulation tasks like focusing, mixing, separation, and sorting. In our institute, we have developed a novel method that utilizes this biocompatible microstreaming to position individual microparticles accurately within a microchannel. The main goal of this research is to analyze the combined flow pattern with precision, determining the direction and strength of the streaming, which is crucial for the proposed particle positioning technique. To achieve this, we employed microparticle tracking velocimetry (µPTV) to study the flow pattern and identify regions of the upward and downward flow. Additionally, we used high-speed particle tracking velocimetry to investigate the timing factors involved in optimizing the programming. This allowed us to visualize the initiation of the superposition of microbubble streaming and Poiseuille flow, as well as the precise alterations in particle trajectory at the moment of piezoelectric activation and deactivation.

Introduction

Over the past few years, there has been a growing demand for non-contact methods to manipulate individual particles, cells, and organisms across various disciplines such as biology, chemistry, engineering, and physics. This increasing need arises from the requirement for precise handling of these entities in numerous applications including detection, focusing, mixing, counting, lysis, and analysis of single cells. In response to this demand, advanced lab-on-a-chip devices have been developed and successfully employed to perform a wide range of functions (Nilsson 2009, Nan L 2014, Sheng W 2014, Mernier 2010, Yang 2006, Nan L 2014, Zhao 2013). Examples such as the enrichment of circulating fetal cells (CFCs), circulating tumor cells (CTCs), and hematopoietic stem cells (HSCs) (Armstrong 2011, Wognum 2003, Bischoff 2003, Y. L. Chen 2014), along with techniques like single-cell electroporation (Khine 2005) and impedance spectroscopy (Han 2006, Cho 2007, J. Z. Chen 2011, Malleo 2010), highlight the significance of cell detection, sorting, and removal. Fur-

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thermore, advancements in micro- and nano-engineering have facilitated the rapid development of various technologies focused on manipulating particles using biocompatible approaches to enhance their effectiveness.

When a microbubble is stimulated by a piezoelectric transducer operating at or near its resonant frequency within a microchannel, it undergoes surface oscillations that are transmitted to the surrounding fluid. This results in the generation of a primary oscillatory flow. In the presence of the microchannel walls, this primary flow induces a secondary flow pattern characterized by counter-rotating vortices. This phenomenon offers a biocompatible and highly efficient method for manipulating cells. By adjusting the frequency and amplitude of excitation, the resulting streaming can be easily controlled, as demonstrated in studies by Ahmed 2016, Rallabandi 2014, C. R. Wang 2013, and A. R. Volk 2019. Recently, the integration of microbubble streaming and Poiseuille flow has gained prominence in lab-on-a-chip devices, enabling a wide range of applications including particle focusing, mixing, separation, and sorting.

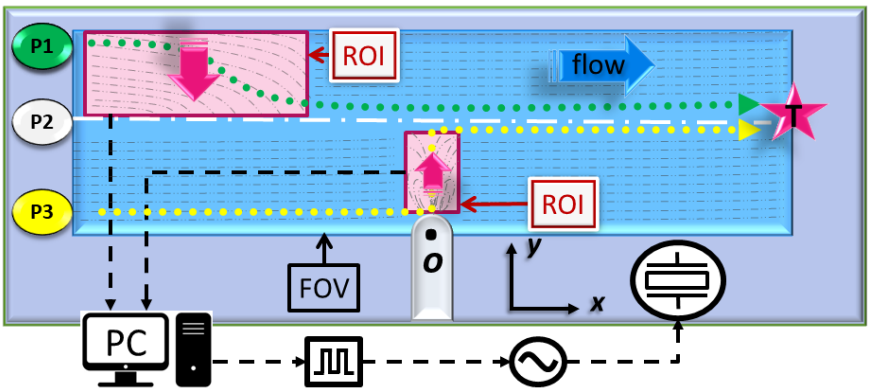

Figure 1: The illustration showcases the process of the single-particle positioning method. Particles located at different lateral positions relative to the target (T) ($y_{p-green} > y_t$ and $y_{p-yellow} < y_t$) enter designated regions of interest (ROIs) that are strategically placed in areas of upward and downward flow. When the piezoelectric transducer is activated, it generates microstreaming, displacing the particles from the ROIs and aligning them with the same spanwise location (y-direction) as y_t. Particles already positioned at the target level $y_{p-white} = y_t$ continue their movement within the Poiseuille flow (without bubble excitation) until they reach the target (T).

In our research institute, we have devised an automated system that employs actuated microbubble streaming to achieve precise manipulation of individual microparticles within a microchannel. This system exhibits notable efficiency and is particularly well-suited for implementation in lab-on-a-chip technology, specifically for tasks such as single-cell analysis, isolation, and removal. Our system capitalizes on the generation of counter-rotating vortices by the actuated microbubble within the microchannel, which induces upward and downward flow, enabling the manipulation and guidance of individual particles towards desired locations. This methodology is succinctly depicted in Figure 1 and further expounded upon in our earlier publications (see A. Bakhtiari 2022 and A. Bakhtiari 2023).

To achieve effective control over particle positioning, it is imperative to gain a comprehensive understanding of the flow dynamics during the initiation and excitation phases. In this study, we utilized µPTV (microscopic Particle Tracking Velocimetry) to quantitatively and qualitatively examine the interplay between Poiseuille flow and microbubble streaming. The primary objectives of this research were twofold: firstly, to determine the direction (upward and

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downward) and magnitude of the microstreaming flow, and secondly, to precisely ascertain the timing and response characteristics of particle movement at the moment of piezoelectric activation and deactivation. These findings play a crucial role in advancing the proposed technique for particle positioning. As such, high-speed particle tracking velocimetry was employed to visually observe the initiation and cessation of microbubble streaming in conjunction with Poiseuille flow. Moreover, it facilitated the meticulous examination of changes in particle trajectory precisely at the specific instances of piezoelectric activation and deactivation.

Experimental setup

The experimental setup comprises a microfluidic system and an optical array, which are situated on a vibration-dampening stage. This design feature serves to attenuate any external vibrations and minimize experimental errors that may arise from such disturbances.

Microfluidic chip

The flow field in a polydimethylsiloxane (PDMS) microchannel was investigated using microparticle tracking velocimetry (μPTV). The microchannel had a height (H) of 100 μm, width (W) of 500 μm, and length (L) of 4000 μm, and it featured a side pit with a width (w) of 80 μm and length (l) of 200 μm (see Figure 2). Another microchannel with similar dimensions but with two side pits spaced 1500 μm apart was also examined. The microchannels were fabricated using the conventional soft lithography technique, following a methodology similar to that described by (C. J. Wang 2012). However, in this study, the projection photolithography method proposed by (Ostmann 2022) was employed, which offers advantages such as low cost and rapid fabrication using readily available and inexpensive components, including masks produced by standard printers.

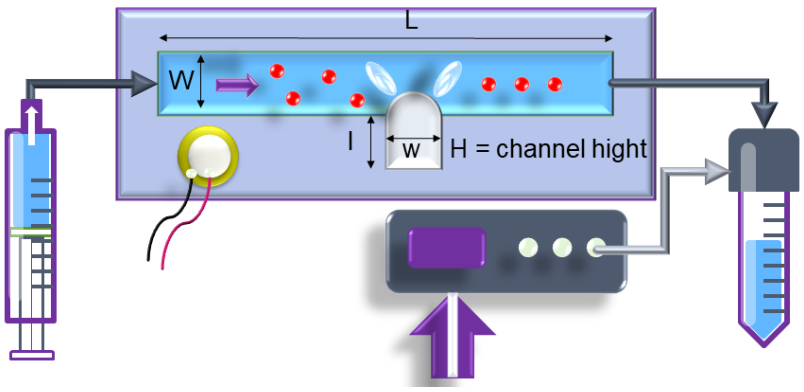

Figure 2 This figure showcases the microfluidic setup wherein the flow parameters are precisely controlled using a syringe pump and pressure controller working together, guaranteeing uniform and stable bubble size.

To maintain experimental reproducibility, the size of the microbubbles was kept constant by controlling the pressure differential between the interior of the channel and the ambient pressure, as proposed by (A. R. Volk 2015).The flow of the aqueous sample solution into the channel was regulated using a syringe pump and pressure regulator (Figure 2) to achieve this pressure control. Further details can be found in the works of A. Bakhtiari 2022 and A. Bakhtiari 2023.

Polystyrene microspheres with a diameter of 2 μm from Microparticles GmbH were used as tracer particles in this experimental investigation. To ensure proper tracking of the particles within the flow dynamics under examination, the density of the particles was adjusted to match that of the fluid, resulting in neutrally buoyant particles. This was achieved by immers-

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ing the microspheres in an aqueous solution containing 23.8 wt % glycerol, followed by vigorous agitation to ensure the uniform dispersion of the microspheres throughout the solution.

Optical setup

The experimental setup consisted of a microfluidic chip placed on a motorized stage, which allowed movement in three dimensions. This stage was integrated into an upright Zeiss AxioImager.Z2 microscope. The microscope was equipped with a dichroic filter and a 10x objective lens (EC Plan Neouar 10x/0.3 M27), which was connected to an sCMOS camera (pco.edge 5.5).

Fluorescent particles were employed in the study, and epifluorescence microscopy was chosen due to its superior signal-to-noise ratio (SNR) compared to bright-field microscopy. Epifluorescence microscopy involves the illumination of fluorescent particles using a continuous-wave laser or high-power LED through the optical path, with the camera capturing the fluorescent light emitted by the particles. Particle detection was achieved by applying filtering and thresholding techniques.

For high-speed particle tracking velocimetry (μPTV), the same setup was used, except that a high-speed camera (v2640) operating at 24 kHz was utilized.

Results

PTV

A set of experiments utilizing General Defocusing Particle Tracking (GDPT) were conducted to track 2 μm tracer particles in a superposition of Poiseuille flow and microbubble streaming. The piezoelectric was actuated at different peak-to-peak voltages v=10~75 V at f=18.9 kHz (Fig.3). The results showed a consistent flow pattern where relatively slow downward flow occurred upstream and downstream of the microbubble, while fast upward flow occurred directly above the microbubble. The downward flow upstream of the bubble (major axis of the first vortex) was observed to converge to the core of the bubble, being faster with a higher gradient than the downward flow downstream of the bubble (major axis of the second vortex). Moreover, particles could be carried to the level at the bubble surface by the upward flow upstream of the bubble, which was not observed downstream of the bubble. The experimental data indicated that the flow pattern partitioned into two primary domains through a separatrix line (highlighted in red). Particles located beyond the separatrix line evaded the vortices and reverted to their original lateral location, nearly identical to their position upstream, without undergoing significant displacement (see Fig.3a and Fig.4a). To improve outcomes in wider microchannels or higher flow velocities, a more efficient approach involving multi-step positioning, with two or more microbubbles in opposing walls, was used. Figure 3b illustrates the μPTV results of a combination of Poiseuille flow and microstreaming using two microbubbles in the microchannel. Even particles that escaped the vortices under the separaterix of the first bubble could be captured by the second counter-rotating vortices. In this scenario, the domain of effective lateral streaming extended (the sum of both microstreamings) across the entire width of the microchannel and could potentially position the particles at any lateral height.

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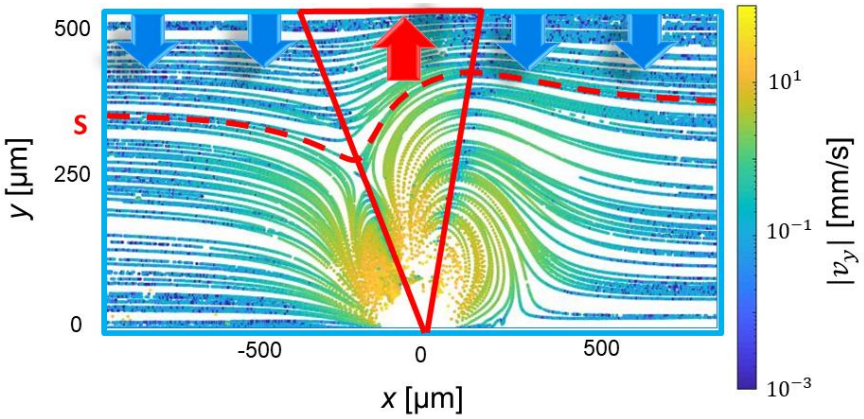
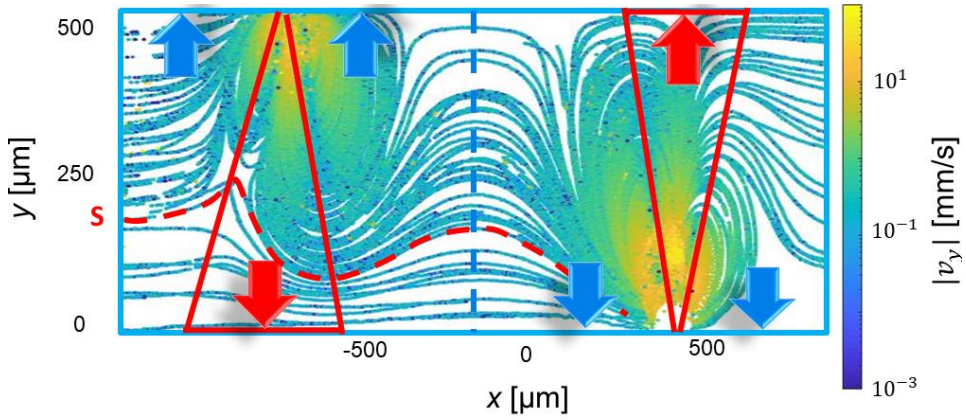


a) One microbubbles



b) Two microbubbles

Figure 3: Streaming measurement of a superposition of Poiseuille flow (from left to right) accompanied by microbubbles streaming at a frequency of 18.9 kHz and a peak-to-peak voltage of 40 V.

Figure 4a depicts the alteration of the separatrix line's lateral level by varying the amplitude of piezoelectric actuation. The reduction in the amplitude from 40 V (Fig.3a) to 30 V results in a diminished ability of the system to manipulate and position particles at a higher lateral position of 125 µm.

Figure 4b illustrates that the separatrix line moves towards the center of the channel with an increase in the peak-to-peak voltage on the piezoelectric element. At a voltage of 75 V, the separatrix line reaches its maximum value, indicating that particles positioned farthest from the desired location can be manipulated effectively using the automatic positioning technique. However, it is crucial to note that excessively high voltages (approximately 140 V in this scenario) can lead to undesirable consequences such as bubble detachment from the cavity, formation of small droplets within the cavity, or damage to the microchip or piezoelectric components.

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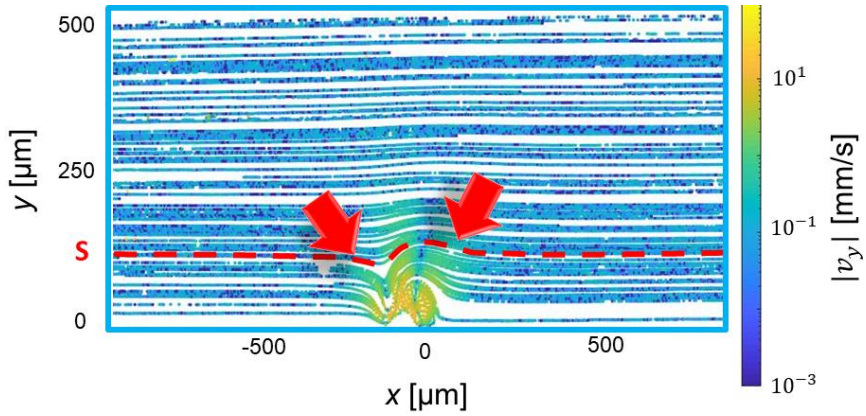
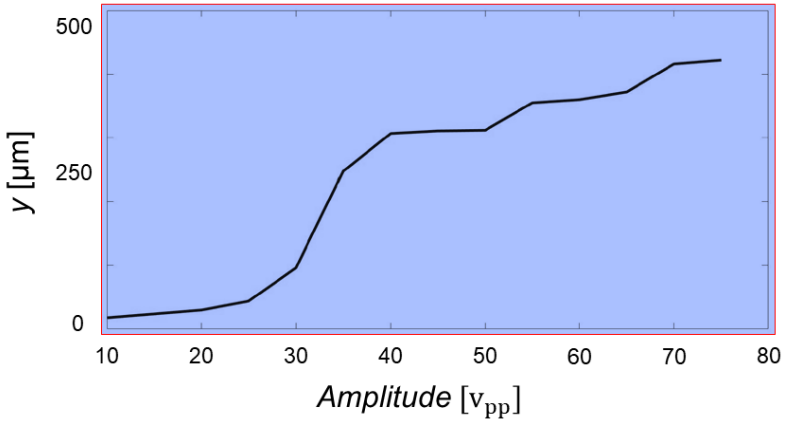


a) Reducing the amplitude from 40 V to 30 V results in a decreased lateral level of the separatrix line.



b) Variation in the separatrix line (S) level across different amplitudes.

Figure 4 Enhancing the separatrix line's extent through amplification of its amplitude.

High-speed PTV

In this study, we employed high-speed microparticle tracking velocimetry (µPTV) to investigate the transition of the flow field from Poiseuille flow to the superposition of Poiseuille flow (from left to right) with microbubble streaming during the activation and deactivation of a piezoelectric element. Figure 5 presents the outcomes of high-speed µPTV conducted over a duration of 3000 images (125 ms) prior to bubble excitation and 10000 images (416 ms) after the initiation of bubble excitation using the piezoelectric element. The images are depicted in black and color, respectively.

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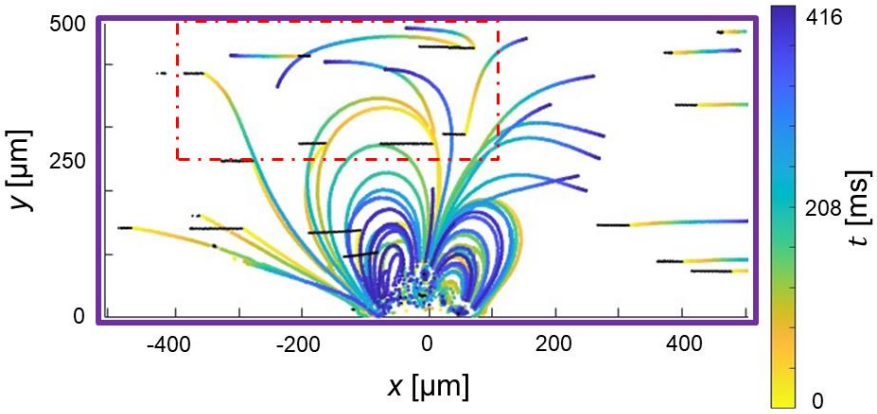
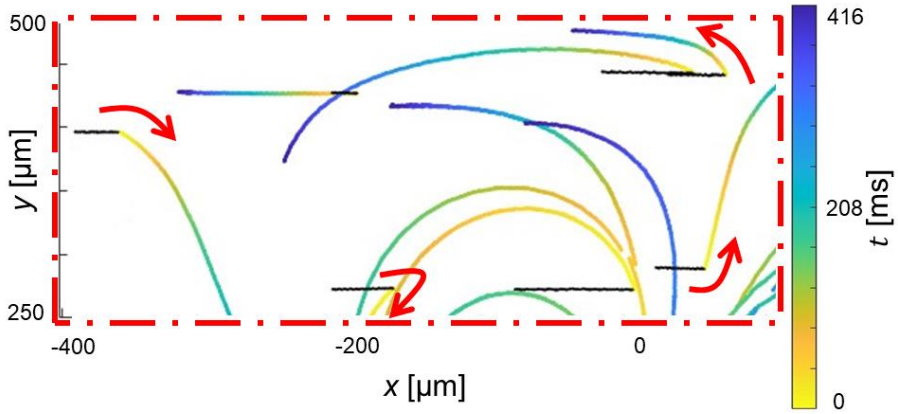


a) Temporal dynamics of particle trajectories: The pre-excitation phase spans a period of 125 milliseconds, while the post-excitation phase extends for 416 milliseconds. These phases are represented in black and color, respectively.



b) The magnified view reveals particles located at an increased distance from the bubble, showcasing synchronized alterations in the trajectories of all particles.

Figure 5 High-velocity micro-particle tracking velocimetry (µPTV) is employed to observe the movement of 2-micrometer particles within the simultaneous occurrence of Poiseuille flow (left to right) and the presence of microbubbles. The tracking is performed at a rate of 24000 frames per second.

A series of experiments were conducted to investigate the behavior of particles in the vicinity of a bubble in a microchannel. Figure 5a illustrates the displacements of particles after the initial cycle of bubble activation. It was observed that all particles experienced immediate changes in their positions. While particles located farther from the center of the bubble exhibited smaller displacements due to slower microstreaming, the alterations still occurred promptly regardless of their proximity to the core vortices (e.g., particles with $x_p < -400$ and $x_p > +400$ µm).

Figure 5b provides a closer examination of the trajectories of particles based on their initial positions. The analysis revealed perfect synchronization of the particles' movements without any noticable temporal delay. This synchronous tracking pattern persisted over a longer period, indicating that the transition from the initial state to the superposition pattern occurred instantaneously without any transition delay that has to be considered.

Similar behavior was observed in the reverse transition from superposition flow to Poiseuille flow. In this case, the Poiseuille flow promptly replaced the superposition flow. Any lateral movement of particles ceased immediately without delay, and the particles remained in their respective lateral positions until they reached the end of the microchannel. The exact final

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positions of the particles can be accurately determined using automated particle positioning techniques. It is important to note that when the Stokes number, which characterizes the response of particles to changes in flow direction, is significantly low (Stokes number <<1), small particles exhibit rapid responses to alterations in flow direction. However, when larger particles with a Stokes number greater than 1 are used, measurements may become more uncertain. This heightened uncertainty should be taken into account during the particle positioning process to ensure accuracy.

Conclusions

Microbubble streaming, triggered by piezoelectric activation near the resonant frequency of microbubbles, offers a biocompatible and efficient strategy for manipulating cells. In this investigation, we have devised an automated system that utilizes microstreaming to precisely position individual microparticles within a microchannel. By employing microparticle tracking velocimetry (μPTV), we have accurately characterized and quantified the flow direction and magnitude in the microchannel under different excitation amplitudes. Our findings demonstrate a consistent flow pattern in all cases, characterized by a relatively slow downward flow both upstream and downstream of the microbubble, alongside a rapid upward flow directly above the microbubble. Additionally, we utilized high-speed particle tracking velocimetry to visualize the combination of microbubble flow and Poiseuille flow during piezoelectric activation and deactivation. Our results indicate an immediate change in particle displacements after the first cycle of bubble activation. Furthermore, when the piezoelectric is deactivated, any lateral particle movement ceases abruptly and without delay, with particles maintaining their lateral positions until they reach the end of the microchannel. This rapid response in altering particle trajectories in response to piezoelectric activation and deactivation makes precise single-particle positioning possible. Using this approach separation, sorting or mixing of particles becomes possible on a microscopic scale.

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