Integrated single camera μPTV and florescence imaging for cell tracking and flow investigation in centrifugal microfluidic devices

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ABSTRACT

A micro-hydrocyclone is investigated as a high throughput particle/cell sorting microfluidic device. A complex flow structure has been reported by limited numerical works that increases the importance of undertaking a comprehensive experimental investigation. In addition to the flow, utilizing damageable bio cells in a micro-hydrocyclone, requires a deep understanding of the interaction between the cells entering the device, the flow structure and its instabilities forming in different operational phases. In such cases the implementation of multiple measurement techniques can be challenging due to the small scale of devices, here < 5 mm diameter. Therefore, for this complex flow an experimental approach is introduced to capture the flow structure and the motion of cells. In this method, tracer particles and fluorescent stained cells are captured simultaneous by a single camera in the same flow field. A custom image processing scheme is used for partitioning cells and particles from the raw data. To determine velocity vectors, μPTV is employed on the segmented data sets to study the effect of flow on trajectory and velocity of each individual cell in the system. By a combined investigation in a vast range of Reynolds number ($50 < Re < 800$) the dynamics of cells are investigated in response to different flow structures and flow regimes.

Introduction

Development of microfluidic applications has led to design and employment of complex devices with complex internal flow organizations. Predicting or enhancing the performance of such devices requires an understanding of its internal flow organization [1]. Optical measurement technics such as particle image velocimetry (PIV) and particle tracking velocimetry (PTV) can be employed to investigate flow transfer in micromixers, particle sorting or separation devices and distinguish the effective and non-effective flow structures forming within a device [2]. The fundamental investigation of flow in microchannels or simple devices has been studied using μPIV [2] for flow in spiral particle separators [3], serpentine micromixers [4] and droplet generators [5]. However, in many of microfluidic applications flow is interacting with an additional cell component. The real time

investigation on the interaction of such components and flow organization can be challenging and has not been investigated in microdevices.

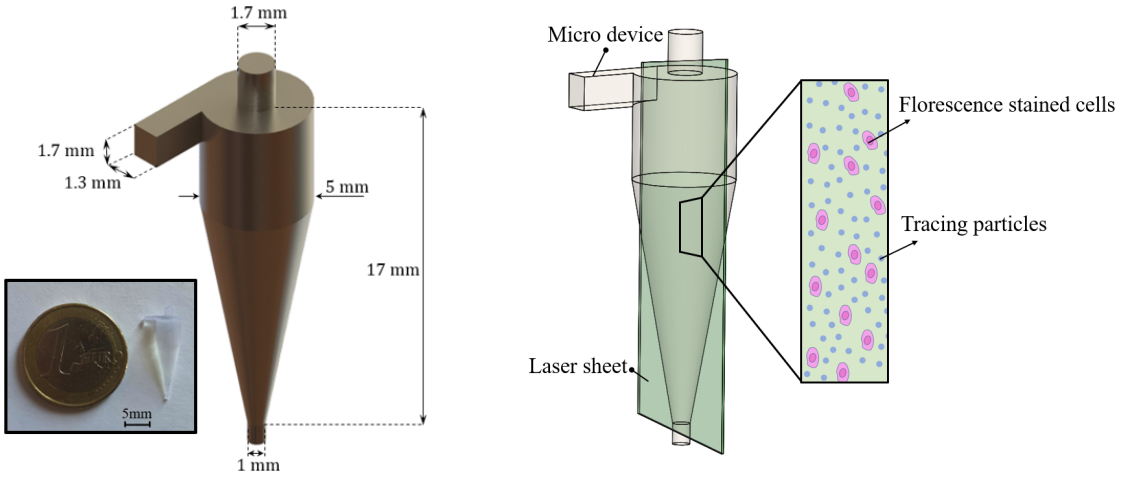
Fluorescent staining and cell tracking in microfluidic devices provides a vast range of information in cell analysis. Labeling the cellular components, facilitates the real-time observation and cell behavior tracking in sorting and separation micro-devices [6]. This method can be used for various cell types, including circulating tumor cells (CTCs), immune cells, and stem cells, investigating their migration, deformation, and interaction behavior [7]. As a result, tracking cells using fluorescent staining in microfluidic devices has become effective in investigating the cell dynamics, with many applications in different fields such as cancer research, immunology, and regenerative medicine [8]–[10].

A hydrocyclone is a centrifugal multi-phase separator widely used in petrochemical, mining and many other industries[11] in large scales. Micro-hydrocyclone technology has been recently introduced to be employed as a high throughput separator without clogging for microfluidics applications [12]. This innovation addresses the low flow rate challenges encountered by microfluidic separators and sorting devices. Instabilities like Dean, Gortler and Taylor-Couette are known to highly effect the performance of the hydrocyclone by increasing the residence time of the particles[13] However, the low operational range of Reynolds number in micro-hydrocyclone leads to a laminar flow suppressing all the effective instabilities [13]. Although macroscale hydrocyclones have been studied for their various applications, the physics of the flow in micro-hydrocyclone is yet to be known.

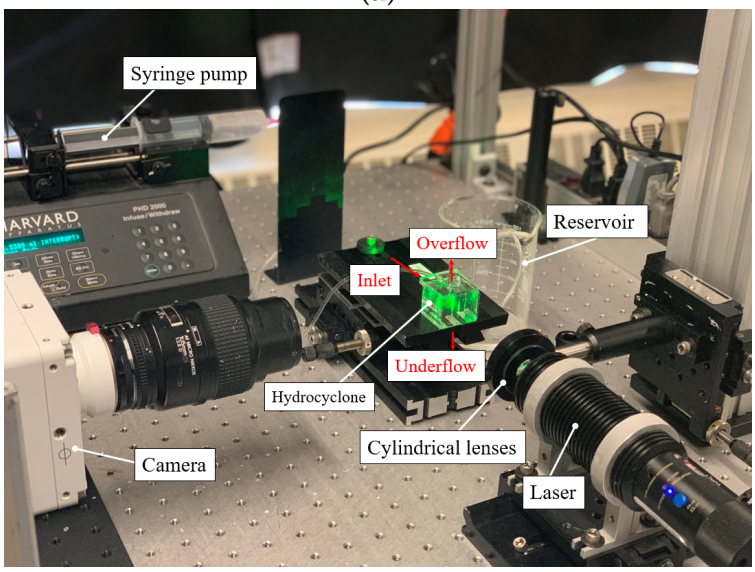
In this research, we present an experimental modified investigation method on a micro-hydrocyclone to simultaneously study the physics of the flow and its interaction with cells in this device. Particle tracking velocimetry (PTV) is applied on the tracer particles to capture the velocity field and the same method is applied on cells to track their trajectory and behavior in the sorting device.

Experimental Setup

The micro-hydrocyclone with $d = 5$ mm as shown in Figure 1(a) was designed with two inlet configurations. The straight inlet is based on a common design, the spiral inlet on the other hand is employed to enhance the performance of the device [14]. An SLA 3D printer (Form 3, FormLab Inc.) with a 25 μm resolution was used to print the inner part of the mold. To increase the surface finishing quality of the cell, the micro-hydrocyclone was coated with a glossy sealing spray. The exterior mold is an acrylic box designed and fabricated to assemble with the 3D printed micro-hydrocyclone.



(a)



(b)

Figure 1 (a) schematic of the microhydrocyclone, (b) Experimental setup used for micro particle tracking in a microhydrocyclone

The microfluidic device was fabricated by molding using a transparent silicone (Solaris™, Smooth-On, Inc.). Its ultra-transparency provides maximum optical accessibility despite the thick sections. The low viscosity of the silicone allows it to flow in and around complex channel geometries easily. A 50.5% carbamide solution is used to match the refractive index of the working fluid with the device and has a dynamic viscosity of 1.905 mPa.s. A PTV setup as shown in Figure 1 (b) was used to apply PTV at the midplane of the micro-hydrocyclone. In this setup a high frame rate camera (Phantom VEO 710, AMETEK Inc.) with a frame rate of 1000 fps was used. A 532 nm laser was used to illuminate the 18 μm hallow glass seeding particles.

Method

Florescence imaging

In most cases cells are transparent by nature and unrecognizable by the optical system. Different kinds of dyes can be used to overcome this limitation and stain a cell based on the targeting cellular structure or component [7]. One of the common approaches is to use florescent dye such as DAPI to make the cell traceable in the existence of laser light [7]. Generating light-emitting cells through the use of fluorescent dyes creates an opportunity of tracing cells utilizing an optical measurement system to investigate their trajectory and behavior [3]. For the early stages of this work in order to increase the repeatability of the experiment and the endurance of the test sample life time LLC Green fluorescent microparticles beads (Cospheric Inc.) are employed mimicking the CTC tumor cells and clusters. Utilizing florescence particles with the same emission wavelength simulates a similar experimental situation that allows to perform a consistency assessment on the method.

Morphological segmentation and

Image segmentation is an image processing technique used to extract and analyze the structures within an image based on their morphological characteristics. It relies on mathematical morphology operations, to highlight and separate objects or regions with specific shapes and sizes. A custom image processing scheme is designed to differentiate and separate cells and tracing particles and generate two sets of data sets for particle and cell tracking as shown in Figure 2. As the first step the raw data from a single camera is preprocessed with noise reduction filters. For each individual frame all regions are scanned, located and categorized based on their shape and size in to particles and cells. Regions marked as cells are erased from the raw data and transferred to a new data set utilizing a background image with the same geometry. Following this step each image will be post processed and prepared for tracking algorithm implementation.

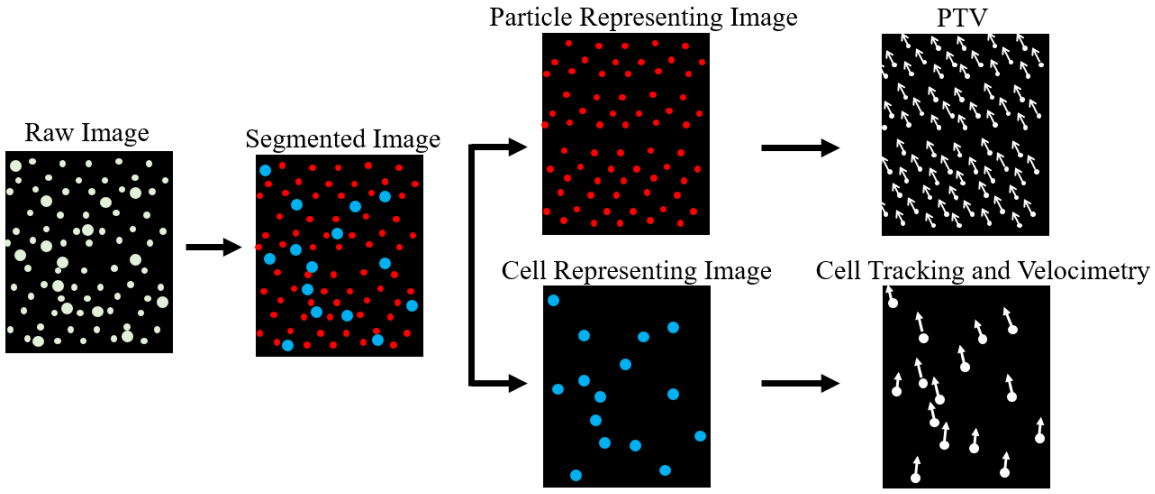


Figure 2 Schematic of image segmentation used to separate cells from partiles for velocimetry.

μ particle\ cell tracking velocimetry

Hollow glass 18 μm particles are used in the refractive index matched working fluid to apply PTV. A commercial code (DaVis V8.4 LaVision GmbH) was used for processing and to calculate the velocity vectors. Background was subtracted from each raw data to enhance the contrast of particles. Particle detection constrains are applied in range of 2 to 6 pixels and intensity threshold is set as 25. The velocity vectors obtained from PTV are dispersed due to random particle placement in each individual image. The resultant velocity vectors are transferred to a structured grid for visualization. The same process will be applied for cell tracking.

Results

Flow on the cross section of a micro-hydrocyclone is presented using μPTV for two inlet configurations. The effect of inlet is investigated on the flow organization in each operational phase of the device.

Streamlines on the midplane of the micro-hydrocyclone in the four different Re are shown in Figure 3. Each case represents a phase in the microhydrocyclone operation. It can be seen in Figure 3 (a) that flow discharges through the spigot. At this phase micro-hydrocyclone is non-functioning as a separation device. With increasing the Re number, a large vortex forms on the midplane of the device as shown in Figure 3 (b). It can be also seen that a low flow rate stream is formed towards the vortex finder results in a low performance separation. Further increase in the Re leads to formation of unstable vortices shown in Figure 3 (c) and a transient phase. At this phase flow passes through both outlets but the number and size of vortices constantly change which can affect the separation performance. In higher Re numbers as in Figure 3 (d) tow stable vortices are generated in the middle of the device height. The location of the maximum velocity, $V^* = {}^{V}/_{V_{max}}$ is also altered from the spigot to two location closer to the center towards both outlets. It indicates the increase in the flow rate passing through the vortex finder at this phase.

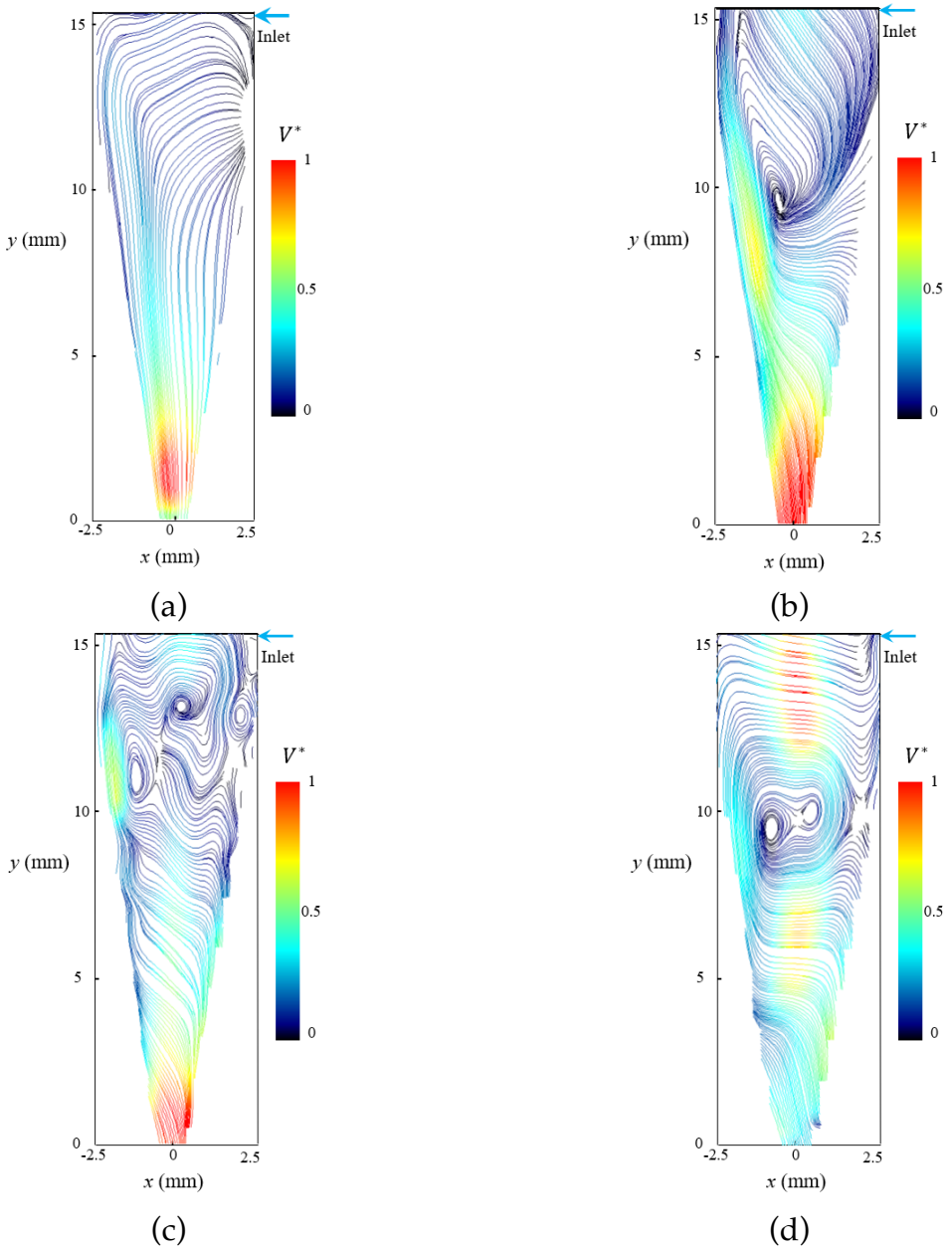


Figure 3 Streamlines on the cross section of the micro hydrocyclone with straight inlet for; (a) $Re < 120$, (b) $Re = 150$, (c) $Re = 300$, (d) $Re > 700$

The alternation on the flow organization entering the micro-hydrocyclone also effects the flow organization in the device. Integrating a spiral channel to the inlet generates counter rotating Dean vortices. The circulating flow entering the micro-hydrocyclone increases the resistance time of the flow in the device and changes its performance. Figure 4 shows the flow organization on the midplane of the micro-hydrocyclone under the effect of a spiral inlet. As can be seen in Figure 4 (a) in a very low Re a weak flow stream is separating and passing through the vortex finder. Increasing the Re in Figure 4 (b)-(d) a strong vortex is formed on upper half off the device. The formation of this vortex indicated the fact that the inlet alternation has led to a larger operational range for the device.

However, it can be seen in Figure 4 (e)that with increasing the *Re* eventually the vortex is shifted towards the vortex finder entrance.

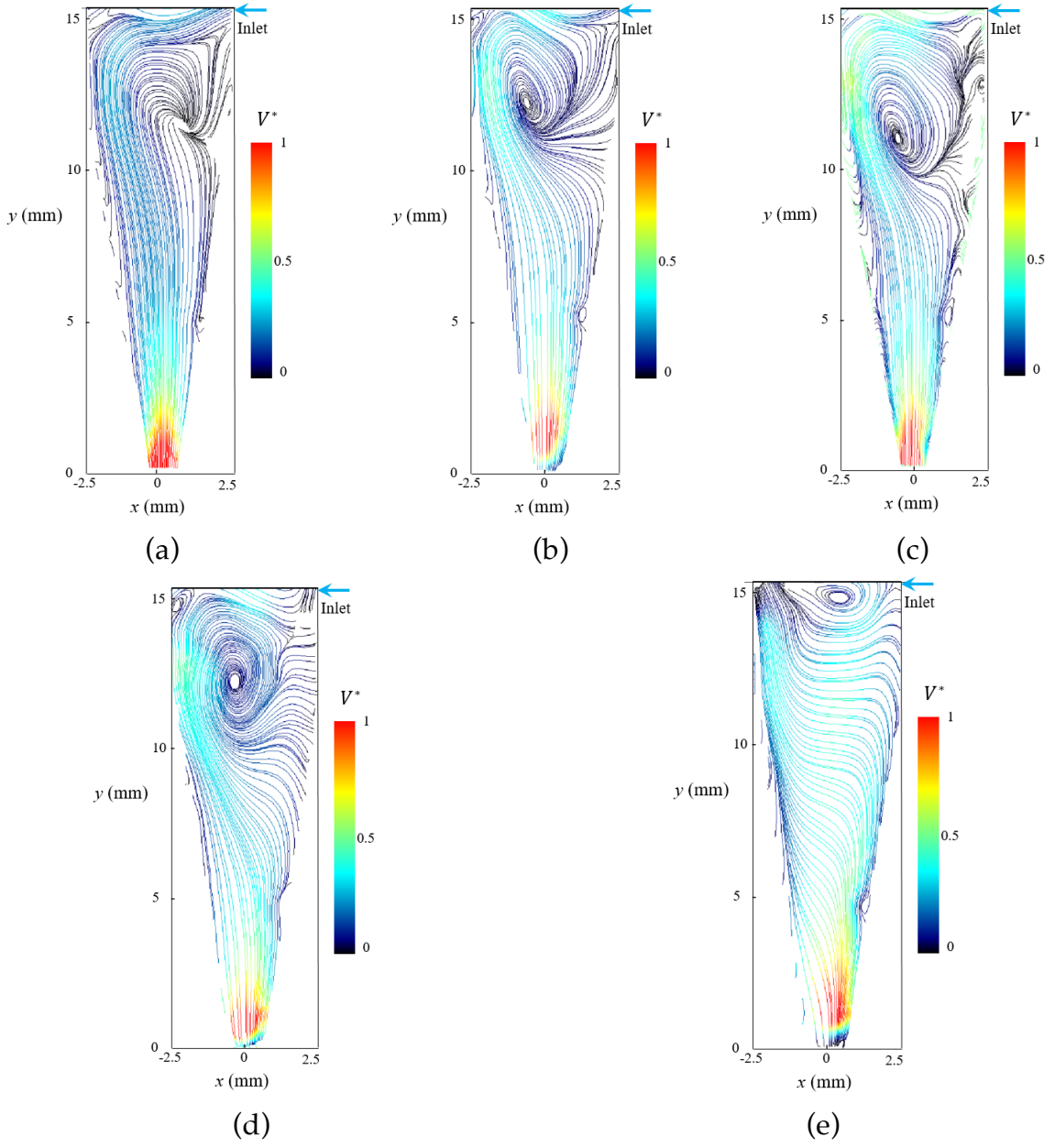


Figure 4 Streamlines on the cross section of the micro hydrocyclone with spiral inlet for; (a) $Re = 35$, (b) $Re = 55$, (c) $Re = 110$, (d) $Re = 150$ (e) $Re = 220$

Axial velocity, V_y^* where $V_y^* = V_y/V_{y,max}$ map on the midplane of a micro-hydrocyclone shows the upward and downward flow motion which are towards the outlets. This map also shows the location of the locus of zero velocity vector (LZVV) $V_y^* = 0$ which is the boundary between the upward and downward motions. The location, elongation and expansion of this boundary effects the separation performance of the micro-hydrocyclone. Figure 5 shows two different cases with the same *Re* number for micro-hydrocyclones with straight inlet Figure 5(a), (b) and spiral inlet Figure 5(c), (d). For the

device with straight inlet axial velocity shows a motion which is towards the spigot but with a spiral inlet where a vortex is generated in the same Re number a region of upward flow motion can be seen.

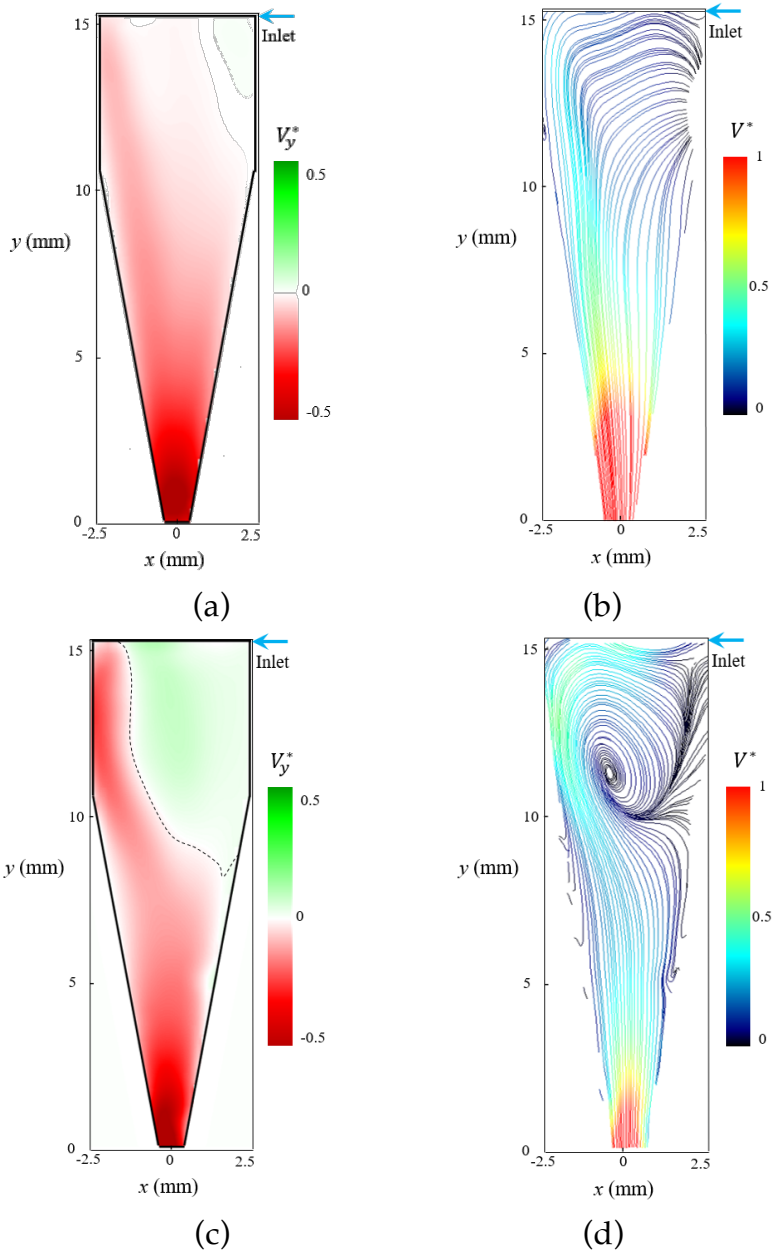


Figure 5 (a)Midplane of a micro-hydrocyclone in $Re = 110$, (a) with straight inlet showing axial velocity (b) with straight inlet showing the streamlines and velocity magnitude, (c) with spiral inlet showing the axial velocity and the location of the LZVV, (d) with the spiral inlet showing the streamlines and the velocity magnitude

Conclusion

The method is proposed to detect the flow velocity and cell behavior simultaneously in the micro device. The image processing scheme separates tracing particles and cell clusters of the image. In

addition to study the effect of flow on the particles this provides the opportunity to investigate the effect of large particles\cell clusters on the flow.

References

[1] A. A. Mouza, C. M. Patsa, and F. Schönfeld, "Mixing performance of a chaotic micro-mixer," *Chem. Eng. Res. Des.*, vol. 86, no. 10, pp. 1128–1134, 2008, doi: 10.1016/j.cherd.2008.04.009.

[2] F. G. Ergin, B. B. Watz, and N. F. Gade-Nielsen, "A review of planar PIV systems and image processing tools for lab-on-chip microfluidics," *Sensors (Switzerland)*, vol. 18, no. 9, pp. 1–23, 2018, doi: 10.3390/s18093090.

[3] N. Nivedita, P. Ligrani, and I. Papautsky, "Dean flow dynamics in low-aspect ratio spiral microchannels," *Sci. Rep.*, vol. 7, no. October 2016, pp. 1–10, 2017, doi: 10.1038/srep44072.

[4] M. Nimafar, V. Viktorov, and M. Martinelli, "Experimental Investigation of Split and Recombination Micromixer in Confront with Basic T- and O- type Micromixers," *Int. J. Mech. Appl.*, vol. 2, no. 5, pp. 61–69, 2012, doi: 10.5923/j.mechanics.20120205.02.

[5] K. I. Belousov, N. A. Filatov, I. V. Kukhtevich, V. Kantsler, A. A. Evstrapov, and A. S. Bukatin, "An asymmetric flow-focusing droplet generator promotes rapid mixing of reagents," *Sci. Rep.*, vol. 11, no. 1, 2021, doi: 10.1038/s41598-021-88174-y.

[6] P. K. Horan, M. J. Melnicoff, B. D. Jensen, and S. E. Slezak, "Chapter 42 Fluorescent Cell Labeling for in Vivo and in Vitro Cell Tracking," *Methods Cell Biol.*, vol. 33, no. C, pp. 469–490, Jan. 1990, doi: 10.1016/S0091-679X(08)60547-6.

[7] Y. Guo *et al.*, "Assessment of the green florescence protein labeling method for tracking implanted mesenchymal stem cells," *Cytotechnology*, vol. 64, no. 4, pp. 391–401, 2012, doi: 10.1007/s10616-011-9417-y.

[8] Y. Yin, Y. Li, Y. Qin, M. Li, G. Liu, and J. Zhang, "Ex-situ experimental study on dynamic behaviors and detachment characteristics of liquid water in a transparent channel of PEMFC," vol. 187, 2022, doi: 10.1016/j.renene.2022.02.031.

[9] A. Jafek *et al.*, "Optimization of Dean flow microfluidic chip for sperm preparation for intrauterine insemination," *Microfluid. Nanofluidics*, vol. 24, no. 8, pp. 1–9, 2020, doi: 10.1007/s10404-020-02366-y.

[10] J. H. Tsui, W. Lee, S. H. Pun, J. Kim, and D. H. Kim, "Microfluidics-assisted in vitro drug screening and carrier production," *Adv. Drug Deliv. Rev.*, vol. 65, no. 11–12, pp. 1575–1588, 2013, doi: 10.1016/j.addr.2013.07.004.

[11] J. Wang, Z. Bai, Q. Yang, Y. Fan, and H. Wang, "Investigation of the simultaneous volumetric 3-component flow field inside a hydrocyclone," *Sep. Purif. Technol.*, vol. 163, pp. 120–127, 2016, doi:

10.1016/j.seppur.2016.02.022.

[12] M. S. Syed, C. Marquis, R. Taylor, and M. E. Warkiani, "A two-step microengineered system for high-density cell retention from bioreactors," *Sep. Purif. Technol.*, vol. 254, no. August 2020, p. 117610, 2021, doi: 10.1016/j.seppur.2020.117610.

[13] G. Zhu, J. L. Liow, and A. Neely, "Computational study of the flow characteristics and separation efficiency in a mini-hydrocyclone," *Chem. Eng. Res. Des.*, vol. 90, no. 12, pp. 2135–2147, 2012, doi: 10.1016/j.cherd.2012.05.020.

[14] Y. Saffar, S. Kashanj, D. S. Nobes, and R. Sabbagh, "The Physics and Manipulation of Dean Vortices in Single- and Two-Phase Flow in Curved Microchannels : A Review," *Micromachines*, vol. 14, no. 12, p. 2202, 2023.