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Hydrodynamic Focusing in Microfluidic Devices

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1. Introduction

In the last decade one of the quickest developing trends in fluid mechanics and chemical engineering has been microfluidics, covering the issues of heat, mass and momentum transfer in microscale. This corresponds directly to intensive research of nano- and microscale technology, as in such scales the system behavior shows significant deviations, compared to macroscale. That is mainly due to a drastically different surface-to-volume ratio and a minor role of buoyancy and inertia forces compared to surface forces like surface tension and adhesion. Due to the characteristic dimensions of microchannels, the flow of liquid is characterized by parallel streamlines, Reynolds number is small and only molecular diffusion is responsible for the inter-diffusion of a reagent.

One of the phenomena involved in a growing number of applications within the microfluidics area is hydrodynamic focusing. Hydrodynamic focusing is a technique relying on squeezing one of the streams in a four-microchannel intersection by two side streams and reshaping it downstream into a thin sheathed film (Domagalski, 2011; Dziubinski and Domagalski, 2007; Mielnik and Saetran, 2006). As can be seen in Fig. 1, the stream of interest, $Q_C$, is focused and sheathed downstream by streams $Q_B$ and $Q_A$.

Index C refers to central inlet, A and B to side streams. Sheet width is denoted by $\delta_s$

Fig. 1. Schematic view of hydrodynamic focusing in a four-channel intersection

By manipulating flow rates of the focusing flows, location of the focused sheet can be deformed and moved out of the symmetry plane. Achieving a precise control of the focused stream width is crucial in various applications of the flow focusing systems.

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2. Applications of hydrodynamic focusing

Due to specific features it has been successfully involved in several microfluidic applications ranging from ultra-fast mixers and microreactors via flow addressed in Lab-on-a-Chip applications and cytometry, two-phase system generators, rheometry and flow visualization to microfabrication. Chemical synthesis in microscale is faster, small volumes and high area-to-volume ratios reduce risks and can improve economics, short diffusion lengths enable fast mixing, generally showing a way for process intensification.

Hydrodynamic focusing is a well known phenomenon in the area of fluid mechanics thanks to Osborne Reynolds, who first used it for flow visualization in his break-through experiment and it is widely utilized as a pipe mixer in chemical technology. However, the first ‘non-academic’ microfluidic application of hydrodynamic focusing was in the area of flow cytometry, a technique for counting, examining and sorting microscopic particles suspended in a stream of fluid. Hydrodynamic focusing, where the core flow of investigated sample is sheathed by an inert fluid, is used in flow cytometry as a way to deliver the sample of suspended cells to the analyzed region in an appropriate form. Such technique is used to precisely align optical detection system giving the possibility of high speed, high through-output analysis easily integrated with sorting, which makes the hydrodynamic focusing the main principle of flow cytometric hardware up to day (Donguen et al., 2005; Givan, 2011; Shapiro, 2003).

Focused stream residing in a channel centre gives a new possibility – to control the focused sheet position by changing the ratio of side streams, which was quickly utilized in the area of µ-TAS (micro-total-analysis systems). In such systems of reactors, mixers and detectors, a precise control of fluid flow is essential. This can be achieved by means of hydrodynamic focusing presenting several advantages as the characteristic switching time being in the order of magnitude of millisecond and near zero dead volume (see the example in Fig. 2).

![Fig. 2. Flow addressing: overall channel design (a), CCD image of focused sample stream (Lee et al., 2005b), visible focused sample stream](image-url)

This idea was developed experimentally, theoretically and by CFD means by several authors (Bang et al., 2006; Brody et al., 1996; Chein and Tsai, 2004; Dittrich and Schwille, 2003; Hyunwoo et al., 2006; Kruger et al., 2002; Lee et al., 2001a; Lee et al., 2005b; Stiles et al., 2005; Vestad et al., 2004;) and it can be used in conjunction with electrokinetic effects (Dittrich and Schwille, 2003; Schrum et al., 1999; Yamada and Seki, 2005). All proposed
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applications used initial pre-focusing prior to precise spatial manipulation of the stream making it possible to integrate a whole system of mixers, reactors and separators at one chip (Chung et al., 2003; Goranovic et al., 2001; Klank et al., 2002; Sundararajan et al., 2004). Such approach is gaining a lot of enthusiasm in analytical science and engineering society as it possesses unquestionable advantages - low sample consumption, possibility of in situ analysis, low cost of single analysis due to mass production lying among the most important ones.

The next area where hydrodynamic focusing is used lies directly within chemical technology field of interest, namely in mixing. The geometrical setup used in cytometry was adopted in a continuous flow mixer leading to laminar, diffusion based mixer which used the hydrofocusing geometry (Knight et al., 1998). The proposed mixer consists of a system of four 10 µm wide channels of rectangular cross section, intersecting in the middle and micromachined in silicon wafer by photolithographic technique. The chip is covered with a glass slip providing the possibility of direct observation of the fluorescent quenching reaction by means of fluorescent and confocal scanning microscopy. Mixing between the inlet and side streams in such a system occurs at the interphase and is fully controlled by diffusion. As the time scale for diffusion changes with the square of a characteristic length, the micron dimensions of focused sheet (down to 50 nm) provides the efficient mixing. In a mixer of such construction, the obtained mixing times are less than 10 µs and reagents consumption is 3 orders of magnitude lower (5 nl/s compared to typical 10 µl/s) than in turbulent continuous flow mixers, which was a big achievement bearing in mind mixing is a challenging issue by itself in micro world.

Such a novel concept of mixer was very flexible and became a subject of many development researches. The speed and efficiency was enhanced by flow segmentation (Nguyen and Huang, 2005), side streams oscillations leading to focused film folding (Tabeling et al., 2004), preventing the slow speed reaction stage which takes place in the intersection, before the focusing process finishes (Park et al., 2006), working on slow reactions requiring steady pumping system (Stiles et al., 2005) or by forcing the turbulence by increasing the flow rate on the other hand (Majumdar et al., 2005) – see Fig. 3.

Advantages of radically quick mixing and low sample flow rate were used immediately in protein folding research. The knowledge of three-dimensional structure of protein and its dynamics is crucial for life sciences. However, the main problem and limiting factor in the observation of such reactions is their time scale being of the order of microseconds. As proposed by Knight et al. (1998), the mixer offers a possibility to change the reaction environment in microseconds; it was adopted to trigger the protein folding due to rapid pH change removing the limiting time scale boundary (Dittrich et al., 2004; Hertzog et al., 2004; Pollack et al., 2001; Russell et al., 2002). The natural step forward for a mixer, integration with a reactor was done. The future development showed more flexibility and advantages of such continuous flow reactor design. Jahn et al., 2004 investigated the possibilities of hydrodynamic focusing application in generation of liposome vesicles. Liposomes, being a class of nanoparticles encapsulating the aqueous volume by phospholipid bilayer, play a crucial role in biotechnology and life sciences delivering drugs or genetic material into a cell. Jahn et al. (2004) took advantage of the laminar character of microfluidic flow, because the lack of temperature, shear stress and composition fluctuations in the reaction environment causes that the product can be characterized by high monodispersity compared to bulk
produced liposomes – important progress bearing in mind that the vesicle size is one of the basic liposome characteristics determining the quantity of encapsulated material.

A – Tabeling et al., 2004; B – Nguyen and Huang, 2005; C – Park et al., 2006; D – Stiles et al., 2005

Fig. 3. Examples of mixing in microchannel

The use of hydrofocusing allows us to create well defined and predictable interphase surface maintaining the fully controlled environment. Such conditions, connected with low inertia typical of microfluidic systems caused by small volumes and laminar flow were used in polymer production. The developed method of continuous fabrication of polymeric microfibers consists in ‘on the fly’ photopolymerization of a hydrodynamically focused coaxial stream (Atencia and Beebe, 2005; Hyun et al., 2006; Jeong et al., 2004) – see Fig. 4.
The main achievement in such technology is flexibility, as controlling the flowrates of sheath and core flow provides a tool to change the fiber diameter and morphology easily – by simple changing the flow conditions the same setup can be used for fabrication of polymeric microcapsules due to break-up of the liquid jet.

Hydrodynamic focusing microreactors can be used in microfabrication and patterning inside the capillaries as well (Kenis et al., 1999; Kenis et al., 2000; Takayama et al., 2001). The idea is to allow the reaction product to interact with the channel wall. Depending on the wall material and reaction product, a wide variety of structures and devices can be generated. Electrodes, wall etches, ridges or lines of crystals can be placed on the walls within the accuracy of 5 µm depending on flow volume rates control. Similarly, such an idea can be used in selective, precise and local treatment of biological cells. As demonstrated by Kam and Boxer (2003), Takayama et al. (2001), Takayama et al. (2003), it is possible to deliver reagents to a cell using multiple laminar streams with subcellular spatial resolution. Similarly, the discussed technique can be used in providing steady, controlled environment for cell population. That can mean equally distributed shear stress (Mohlenbrock et al., 2006) or stable in time, predictable and homogeneous chemical environment for lysis (Sethu et al., 2004). The applications of precisely controlled laminar fluid layers were also presented as a technique for fabrication of advanced membranes.

The characteristic features of hydrodynamic focusing can be used in rheology (Waigh, 2005; Wong et al., 2003). Diluted polymer particles delivered and focused precisely in the channel centre experience deformations due to shear forces and elasticity. Such an isolated molecule in the focused stream has a determined position in transverse axis and forced orientation parallel to flow direction. Labeling the endings of polymer chain by fluorescent probes allows one to observe a single molecule dynamics and its response to changes in flow conditions. Dynamics of such a single molecule can reveal complex rheological properties providing deeper insight into fundamental issues comparing to bulk rheological measurement.
The hydrofocusing geometry is an example how a channel modification, channel intersection can result in complication of physical phenomenon, implicating the possible applications. The geometry consisting of intersection of four rectangular cross section channel has found application also in two-phase flow. Many authors (Anna et al., 2003; Caubaud et al., 2005; Cristobal et al., 2006; Dreyfus et al., 2003; Joanicot and Ajdari, 2005; Garstecki et al., 2005a; Garstecki et al., 2005b; Raven et al., 2006; Seo et al., 2007; Utada et al., 2005; Ward et al., 2005; Xu and Nakajima, 2004;) used this geometry in a straight form or modified by a nozzle after the intersection to produce monodisperse two-phase systems (cf. Fig. 5).

Fig. 5. Examples of generation of two-phase system in microchannels

Recently, hydrodynamic focusing has been applied in micro-PIV as a selective seeding technique (SeS-PIV) (Blonski et al., 2011; Domagalski et al., 2006; Domagalski et al., 2007; Domagalski, 2010; Domagalski, 2011; Mielnik and Saetran, 2006). Particle image velocimetry (PIV) is a flow visualization technique, where a flow velocity field is deducted from the displacement of tracer particles moving with investigated medium over time intervals. Simplifying, the flow field can be determined by correlating the tracer displacement on sequential frames. Due to small dimensions, in opposition to standard PIV technique where light is introduced in the form of a laser, in µ-PIV the flow is subject to bulk illumination to evade the technical problems with light alignment and light sheet generation. In SeS-PIV, a modification of µ-PIV, the tracer particles are introduced in the hydrofocused sheet making the width of the sheet responsible for the resolution (in opposition to the focal depth of optical system in the case of standard µ-PIV). That can de-bottleneck the system, making the measurement less dependent on the optical setup and permitting highly depth-resolved and instantaneous (time-resolved) velocity field measurements.
3. Three-dimensional aspect of hydrodynamic focusing

As was shown before, a lot of work concerning hydrodynamic focusing had been done and many examples of applications exist in the literature. However, little is known about this phenomenon on a basic level. So far, the papers on hydrodynamics of fluid focusing in microchannels present theoretical approach based on analytical solution of the flow in a rectangular cross-section channel (Chen et al., 2006; Solli et al., 2006; Wu and Nguyen, 2005 a,b,c).

The last publications, however, show the complexity of this phenomenon. A detailed investigation of the three-dimensional structure of hydrodynamic focusing performed by means of CLSM (confocal laser scanning microscopy) reveals two aspects of stream deformation (Blonski et al., 2011; Domagalski et al., 2007; Domagalski, 2011; Domagalski and Dziubinski, 2010; Dziubinski and Domagalski, 2010). The first one consists in a non-uniform distribution of stream width and the second one relies on an additional curvature of the focused stream while pushing it away from the channel axis by non-symmetric side streams.

Experimental investigations indicated that in the case of symmetric side streams focused flow sheet was not necessarily uniform and its thickening close to the walls of a microchannel might reach undesirable values. A three-dimensional study of this effect confirmed that the focused sheet was not flat and with an increasing flow rate it exhibited nearly tripled thickness at both side walls, see Fig. 6.

Fig. 6. The 3D projection of confocal microscopy image of hydrodynamic focusing with cross-section a,b,c - thickening of the focused plane close to side walls observed when increasing flow rate ($Q_A/Q_B = 1$; $Re= 3.28; 6.46$ and $12.92$, respectively)

A detailed flow pattern analysis revealed possible regimes of focused stream shape: barrel-like shape, characterized by a decrease of width towards the top and bottom walls, Fig. 6a, flat uniform shape, Fig. 6b, and double concave shape, Fig. 6c.

Analyses of available experimental data reveal three regimes of the flow-focusing mechanism depending on the value of Reynolds number:

- at $5<Re<8$ a nearly flat focused plane with constant width can be obtained,
- $Re<5$ creates a slightly convex shape of the focused streams,
at Re > 10 double concave shape is present, complete layering of the focused flow on the side walls takes place at the Reynolds number approaching 50.

Figure 7 show shapes of the focused stream obtained by CFD simulation for channels of rectangular cross section 300 × 400 μm. The geometry and dimensions of the CFD model were identical to the experimental device. The boundary conditions were set as mass flow at the inlets and pressure at the outlet of microchannel (Blonski et al., 2011).

Focusing ratio – the ratio of flow of the focused flow to the sum of focusing streams $Q_c/(Q_A+Q_B)$

A numerical simulation performed for four different focusing ratios indicates that thickness of the focused plane decreases with an increase of the focusing ratio, Fig. 7. However, a very strong effect on the focused sheet structure is observed by varying total flow rate (the flow Reynolds number), see Fig. 7 and 8. Increasing the Reynolds number above 10 practically destroys the flow focusing mechanism and for the Reynolds number above 50 the focused liquid is fully layered on the top and bottom walls, being absent from the channel center. Numerical calculation confirms very well the experimental data.

Fig. 7. Distribution of the focused liquid for three different Reynolds number (rows) and four different focusing ratios (columns)
Description of the shape of focused stream is complicated in the case when the focused stream is pushed away from the channel axis by non-symmetric side streams. Such behavior of the focused stream is shown in Fig. 9 which presents images of stream projection obtained by means of confocal microscopy with corresponding CFD simulations (Domagalski, 2011).

When the stream is pushed away from the channel center, the previously described deformation in the form of uneven width of the stream is overlapped by the next deformation in the form of stream curvature perpendicular to flow direction. This behavior is confirmed by CFD simulations, as shown in Fig. 10 which presents a comparison of...
relevant cross sections obtained experimentally by the confocal microscopy with results of the CFD simulations for a channel cross section $1020 \times 800 \mu m$.

Fig. 10. The comparison of experimentally determined shape of focused stream (upper diagram) with CFD modeling (bottom diagram) for different values of side streams ratio $Q_A/Q_B$: a) 1.0, b) 1.73, c) 2.0, d) 3.0 and e) 7.56

This figure makes it possible to compare directly the shapes of deformed stream, showing good agreement of CFD simulation result and the observed system behavior.

4. The effect of properties and velocity of flowing media and channel size on the shape of focused stream

A very significant aspect of designing and operation of the systems based on hydrodynamic focusing is to determine the position of focused stream inside the outlet channel in given conditions of flow. As it has been stated earlier, when the flow is focused by identical side streams $Q_A=Q_B$, the focused stream leaves a microchannel flowing in the center of the outlet channel. In the case when the focusing streams are not symmetric, the focused stream is pushed away from the channel axis (Domagalski, 2007; Domagalski, 2008; Domagalski, 2011).

The basic geometric parameters that characterize the shape and position of stream in the microchannel were displacement of the stream from the center of channel axis $z$ and its curving represented by distance $c$ which is the difference of displacement of central part of the stream and its near-to-wall part, cf. Fig. 11.
For example, Figure 12 shows dependence of the position of focused stream in the outlet channel on the ratios of side flow rates for the system of channels with cross sections $1020 \times 800\mu m$ and $260 \times 200\mu m$ (Domagalski, 2011).

![Diagram of microchannel cross sections](image)

Fig. 11. The basic geometric parameters characterizing the shape and position of stream in a microchannel

Channel cross section: 1020×800 μm (left-hand side diagram), 260×200 μm (right-side diagram)

![Graphs of stream displacement](image)

Fig. 12. Displacement from the centre of channel axis $z$ as a function of the side stream ratio $Q_A/Q_B$

These diagrams have a characteristic point with coordinates $A(1,0)$ corresponding to the variant of symmetric focusing when volumetric flow rates of side flows are the same, and horizontal asymptote corresponds to a physical border in the form of the channel wall. It can easily be observed that pushing the focused stream away from the microchannel axis grows with an increase of the ratio of side streams.

Another aspect of deformation of the focused stream is the dependence of stream curvature on flow conditions. Figure 13 shows the dependence of curvature $c$ of the focused stream on pushing the stream away from the channel axis $z$. 

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As follows from the figures, curving of the focused liquid stream pushed away from the microchannel axis increases with the process of pushing it away. Additionally, this effect is enhanced when the velocity of media flowing through the outlet channel grows.

While comparing diagrams shown in Fig. 12 and 13 one can observe that the relations have a similar character irrespective of the channel cross section. To investigate whether it is possible to exclude the effect of the channel cross section on the characteristics of hydrodynamic focusing, it was proposed to use dimensionless values. For this purpose the dimensionless pushing of the focused stream away from the channel center $z'$ and dimensionless curving $c'$ was used. These values are defined as follows:

$$z' = \frac{z}{D_z}$$  \hspace{1cm} (1)

$$c' = \frac{c}{D_z}$$  \hspace{1cm} (2)
where: $D_z$ – equivalent channel diameter, $z$ – pushing away from the channel axis, $c$ – channel curvature.

In the case of a channel with rectangular cross section of dimensions $a \times b$, the equivalent diameter has the form:

$$D_z = 4 \frac{A}{O} = \frac{2ab}{a+b}$$  \hspace{1cm} (3)

where: $A$ – cross section of the channel, $O$ – channel perimeter flown by liquid, $a$ and $b$ – channel dimensions.

To investigate the effect of the channel cross section on hydrodynamic focusing, the cases of similar hydrodynamics were taken into account, and the criterion of similarity was the Reynolds number defined by the equation:

$$Re = \frac{u D_z \rho}{\mu}$$  \hspace{1cm} (4)

where: $u$ – liquid velocity, $\rho$ – liquid density, $\mu$ – liquid viscosity

The effect of channel dimensions on pushing the focused stream away from its axis as a function of the ratio of side stream flow rates for different values of the Reynolds number is illustrated in Fig. 14, while the effect of the channel dimension on the dimensionless curvature $c'$ is shown in Fig. 15.

Fig. 14. Dimensionless pushing of the focused stream away from the channel axis as a function of the ratio of focused streams for different values of the Reynolds number (left-hand side diagram) and for channels of different dimensions (right-hand side diagram)

From the analyses of experimental data shown in Fig. 14 and 15, it follows that the dimensionless value of pushing the focused stream away does not depend on the channel dimensions, while the dimensionless curvature of the focused stream $c'$ is dependent on the channel dimension. Curving of the focused stream is bigger in a smaller microchannel.
Fig. 15. Dimensionless deformation of focused stream $c'$ as a function of dimensionless pushing off from the channel axis $z'$ at constant Reynolds number $Re=8.1$

To estimate the position and shape of focused stream the following form of correlation equations was proposed:

$$z' = f(Q_A/Q_b)$$

$$c' = f(z', Re, D_z)$$

Preliminary knowledge of the shape of stream focused in given flow conditions is a basic information while designing and operating the devices in which hydrodynamic focusing is applied.

Based on available experimental data, the following form of Equations (5) and (6) is proposed

$$z' = -0.955 (Q_A/Q_b)^{-0.184} + 1.04$$  \hspace{1cm} (7)

$$c' = 0.027 z' Re - 0.0393 (D_z/D_{ref}) + 0.0243$$  \hspace{1cm} (8)

A reference diameter $D_{ref}$ is assumed to be 1000 $\mu$m which is the upper limit of dimension of the channel defined as a microchannel.

A comparison of experimental data with the values calculated using correlation equations (7) and (8) is shown in Fig. 16.

Using Equations (7) and (8) it is possible to determine the position and shape of focused stream with a maximum error reaching 25%. The equations are valid equally for channels of dimensions ranging from $260 \times 200$ to $1020 \times 800$ $\mu$m and for media with the following parameters: density 998 to 1097 kg/m$^3$, viscosity 0.997 to 12.5 mPas, surface tension 31 to 73 mN/m and for the range of liquid flow rate in the microchannel corresponding to the range of Reynolds number from 4.5 to 14.
To investigate the effect of surface tension and liquid viscosity on the parameters of focused stream, measurements were taken for liquid with the surface tension 31 mN/m\(^2\) (water with surfactant Triton X-100, Sigma-Aldrich) and liquid of viscosity higher than that of water amounting to 12.5 mPas. Results of exemplary measurements are given in Fig. 17 and 18.

Based on the investigations it can be claimed that in the used range of measurements the effect of media properties on the character of a hydrodynamic phenomenon of stream focusing in the microchannel is negligibly small.
5. Modification of micro-PIV flow visualization technique using hydrodynamic focusing phenomena

Micro Particle Image Velocimetry (micro-PIV) is a flow visualization technique for microfluidics (Raffel, 2007), where a flow velocity field is constructed from the displacement of tracer particles moving with investigated medium over time intervals. Simplifying, the flow field can be determined by correlating the tracer’s displacement on sequential frames. Due to small length scales of the observed phenomena, the flow is a subject of volume illumination, meaning the whole channel volume is illuminated and the measurement is based on focal depth of optical system, as only tracers within the depth of focus are clearly visible. That caused several problems as particles from below and over the focal plane participate in image brightness as background noise and evidently depreciate the evaluation accuracy.

To overcome this drawback, low concentration of tracers is usually used and the performed averaged correlation procedure averages the results over a large number of pairs of images, this however costs time.

Recently, the flow focusing method was proposed to introduce the tracers as a thin layer instead of whole volume seeding (SeS-PIV Selective Seeding PIV) (Mielnik and Saetran, 2006; Domagalski et al., 2008; Blonski et al., 2011; Domagalski, 2011). Such layer can be obtained in a rectangular cross-section channel via hydrodynamic focusing, which is shown in Fig. 19. Limiting seeding to a thin layer improves spatial resolution of the velocity field evaluation and permits to apply higher tracers concentration, hence allowing for acquisition of shorter sequences of images.

As visible, the stream containing tracers is squeezed by tracer-free side streams, which makes it possible to create the confined, narrow layer of tracers. Now, two conditions are necessary to take the advantage of such a setup. First, the flow has to be laminar, so the focused stream will not be perturbed, second, the diffusion effect has to be negligible. The first condition is generally fulfilled in a micro-area – with sub-millimeter characteristic...
length and liquid flow that is usually true. The second condition is dependent on tracer particle diameter, as the diffusion speed is proportional (Einstein-Stokes formula) to particle diameter.

Fig. 19. The classical micro-PIV (A) and the idea of its modification as SeS-PIV (B)

Assuming that the focused stream is narrower than the depth of focus of a microscope, the measurement becomes independent of optical parameters. Due to limiting the source of fluorescent light to well-defined thin surface, the signal to noise ratio is strongly improved – see Fig. 20.

Fig. 20. Signal to noise ratio S/N as a function of test section depth (correlation depth)

What is more important, such a modification eliminates the seeding concentration limit, allowing the tracer layer to seed densely, resulting in the possibility of analyzing flow field on the basis of reduced (compared to standard, low concentration, volume illuminated micro-PIV) number of image pairs. The measurement can take less time, allowing for the measurement of non steady flows.
The whole depth section of the flow contributing to the measured velocity field is called the depth of correlation. Physically it is the depth of focus of the microscope, extended by the effect of diffraction and tracer particles geometry (Meinhart et al., 2000).

At the first step of analysis it is useful to compare the raw images of micro-PIV and its modification SeS-PIV. The comparison shown in Fig. 21 reveals a visible quality increase in the case of the SeS-PIV method.

Fig. 21. Comparison of SeS-PIV image (upper) and micro-PIV raw images (bottom)

One may find better contrast of the image with focused seeding. This is caused by the lack of background, as no tracers are present outside focused, controlled tracer streams of known geometry. Moreover, the tracers are flowing in a thin layer, thinner than the depth of focus, so their images lack the diffraction rings as opposed to out-of-focus particles present in the micro-PIV picture. The visible blurred area near the obstacle in SeS-PIV picture is due to the three-dimensional deformation of focused stream. During flow over the ridge casing the tracers are coming out of the depth of focus. However, this effect was observed only at the highest tested velocities.

The velocity profiles determined by micro-PIV and SeS-PIV methods are presented in Fig. 22 (Blonski et al., 2011).

5.1 Applicability of the novel SeS-PIV technique

Applicability of the presented measuring technique is related directly to focusing hydrodynamics, the shape of cross section of the focused stream and diffusion of tracers used in the measurements. This applicability is limited by deformations of the focused stream and tracer diffusion rate.

The limit of acceptable deformation of the stream is determined by its curvature not exceeding the depth of correlation. The main mechanisms deforming the focused stream are Dean vortices, Moffat vortices and diffusion (Dascopoulos and Lenhoff, 1989; Domagalski, 2009; Ismagilov et al., 2000; Kamholtz et al., 1999; Kamholtz and Yager, 2001; Kamholtz and
Dean vortices formed as a result of unbalanced centrifugal force during motion along the curved line play a key role in deformation of the stream, inducing an increase of the cross section of the focused stream in near-to-wall regions. Intensity of the Dean vortices is characterized by the Dean number which is directly proportional to the Reynolds number (Munson et al., 2002). The effect of Dean vortices decreases with a decrease of the Reynolds number which is illustrated by rectangular cross section of the stream at Re=5. Transition into the region Re<1 (particularly in the creeping flow regime Re « 1) totally neutralizes the effect of Dean vortices on flow in the microchannel but causes generation of Moffat vortices (Mercer, 2004; Moffat, 1964). These are the structures formed due to wall action in the immediate vicinity of stagnation points. However, they do not have such a destructive effect on the stream shape as the Dean vortices have.

The effect of diffusion depends strongly on the particle size of tracers used in measurements. For a typical tracer size d=2 μm, diffusion coefficient is of the order D=2.2 \times 10^{-13} \text{m}^2/\text{s}. In this case the path of diffusion is of the order of several micrometers along the whole length of a typical microchannel. Since the width of focused stream is much bigger, the effect of diffusion on the accuracy of measurements is negligible. However, in the literature there are examples of researches carried out with the use of quantum dots of size 20 nm, which much enhance the process of diffusion and do not allow us to abandon its effect on the shape of focused stream.

Hydrodynamic focusing of a liquid stream provides an opportunity to control the position of focused stream in the outlet channel. This technique used to illustrate velocity fields, enables mapping of the velocity field in subsequent channel cross sections (at different positions of the stream in the outflow channel), and consequently allows velocity to be measured in the entire liquid volume. The limit of applicability of this technique is determined by curvature of the focused stream which cannot be bigger than the depth of correlation of micro-PIV. Deformations limiting in this way applicability of the hydrodynamic focusing to a modification of the micro-PIV technique are primarily the...
functions of the Reynolds number, and for the non-symmetric variant of focusing also the ratio of velocities of the flowing media.

Concluding, it should be stated that the applicability of the SeS-PIV method is determined by three conditions:

1. The Reynolds number for the outlet stream is below Re=10.
2. The diffusion path is much smaller than the width of focused stream.
3. The width of focused stream is smaller than the depth of correlation of the microscope.

The proposed correlation equations (7) and (8) are used to estimate preliminarily the shape and position of focused stream inside the channel which enables determination of the region in which condition 3 is satisfied, and consequently enables quick identification of the applicability of the SeS-PIV method.

6. Conclusions

This chapter presents a review of applications of hydrodynamic focusing and the latest research in this area. Hydrodynamic focusing being a well-established technique in microfluidic area has found many applications. Due to specific features it has been successfully involved in several microfluidic applications ranging from ultra-fast mixers and microreactors via flow addressed in Lab-on-a-Chip applications and cytometry, two-phase systems generators, rheometry and flow visualization to microfabrication. Chemical synthesis in microscale is faster, small volumes and high area-to-volume ratios reduce risks and can improve economics, short diffusion lengths allow for fast mixing, generally showing a way for process intensification.

The latest researches, however show precisely a new complicated three-dimensional aspect of this phenomenon indicating novel promising possibilities of future applications and development. A detailed investigation of the three-dimensional structure of hydrodynamic focusing performed by means of CLSM (confocal laser scanning microscopy) reveals two aspects of stream deformation. The first one consists in a non-uniform distribution of stream width and the second one relies on an additional curvature of the focused stream while pushing it away from the channel axis by non-symmetric side streams. The influence of properties and velocity of flowing media and channel size on the shape and position of focused stream in the microchannel has been presented.

A modification of the micro-PIV technique by introducing the tracers in hydrodynamically focused thin layer instead of volume seeding was proposed. Such modification known as SeS-PIV improves the raw image quality by removing the background noise ratio and permits higher seeding concentration. These features drastically improve the analysis of raw images – comparing to micro-PIV technique, making SeS-PIV techniques a valuable tool for microfluidic flow visualization.

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Advances in Microfluidics provides a current snapshot of the field of microfluidics as it relates to a variety of sub-disciplines. The chapters have been divided into three sections: Fluid Dynamics, Technology, and Applications, although a number of the chapters contain aspects that make them applicable to more than one section. It is hoped that this book will serve as a useful resource for recent entrants to the field as well as for established practitioners.

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