Characterization of Microfluidic Devices by Measurements with μ-PIV and CLSM

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Abstract. Microfluidic devices are successfully in use for several applications in chemical engineering and biotechnology. Nevertheless, there is still no breakthrough for microprocess engineering because of a huge lack in understanding of the mechanisms on microscales for momentum transfer, hydrodynamics and mass transfer. Some important questions concern the design of a junction to reach acceptable mixing qualities with minimum pressure drop and narrow residence time distribution even under laminar flow conditions. The micro-particle image velocimetry (μ-PIV) in conjunction with confocal laser scanning microscopy (CLSM) have been used for the characterization of momentum and mass transfer at the Institute of Environmental Process Engineering to evaluate microfluidic devices. The calculation of three-dimensional flow and concentration fields is possible with two-dimensional measurement data for common stationary cases. Streamlines out of velocity gradients and isosurfaces out of fields of the same concentration are providing a helpful impression of the performance of microdevices based on highly reliable measurement data. A quantitative analysis of the velocity and concentration fields allows the calculation of residence-time distribution and mixing quality, which enables the adjustment of microreactor geometries for the demands of chemical, and biochemical reactions.

1 Introduction

Process intensification plays a key role in saving resources, which is one of the main goals in sustainable development. Microfluidic devices are a very interesting tool for process intensification because the contact time between educts is exactly adjustable, backmixing is negligible and the heat transfer for temperature-sensitive reactions is easy to control. Over the past decade, micromixers have been developed for a broad range of applications, such as bioanalytical techniques or the production of organic compounds. The growing demand for flexible multipurpose plants opens a wide field of application for continuous processes in microstructured devices [1]. The design of modern technical applications, e.g., reactions with high selectivity, a precise analysis of local mass transfer and hydrodynamics for different flow regimes, requires microscopic measurements of flow and concentration fields. Although microreaction technology offers diverse possibilities for optimizing processes with highly selective reactions this capability remains largely unused. *Wong* et al. [2] recently gave a general overview of flow phenomena and

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mixing characteristics in T-shaped micromixers and showed the tremendous demand for further experimental data on flow and concentration fields on microscales.

In the last decade particle image velocimetry (PIV), as a nonintrusive technique for measuring flow fields, has been successfully adapted for measuring flow fields within microfluidic devices with micrometer-scale resolution, e.g., [3]. Recently, this nonintrusive diagnostic technique was extended to the infrared waveband [4] with the advantage that measurements can be made directly through silicon without the need for an optical access. For higher temporal resolution, *Shinohara* et al. [5] used a high-speed μ-PIV technique by combining a high-speed camera and a continuous-wave laser in order to investigate transient phenomena in microfluidic devices. Recently, a confocal laser scanning microscope (CLSM) in conjunction with micro-particle image velocimetry (μ-PIV) has been developed by using a dual high-speed spinning disk. While the upper disk is a rotating scanner that consists of 20 000 microlenses, the lower one is a Nipkow disk that consists of 20 000 matching pinholes of 50 μm in diameter. This system shows an optical slicing capability with a true stepwise-resolved μ-PIV vector-field mapping [6]. Another new development is the stereoscopic μ-PIV with an epifluorescence stereo microscope and two synchronized CCD cameras. *Lindken* et al. [7] applied a stereoscopic μ-PIV in the investigation of the three-dimensional flow in a T-shaped mixer.

Although the experimental data of flow velocities in magnitude and direction are essential for calculation and validation of numerical simulations, only a combination of flow and concentration fields enables a full analysis and understanding of phenomena as well as the analysis, development and evaluation of novel microfluidic processes. For direct visualization of flow structures in microchannels, the injection of dye to color a part of the flow field is a common method. As dyes, regular fluorophores are normally used, as their emissions can be stimulated by laser light (e.g., [8]). Contrary to macroscopic flows, it is not practicable to use a thin laser lightsheet because of the tiny dimensions (down to several micrometers) in microdevices. On a microscale, it is essential to illuminate the complete microchannel volume. *Sinton* [9] did a comprehensive study of microscale flow visualization techniques. Recently, *Matsumoto* et al. [10] presented an optical measuring technique for concentration fields in microchannels by averaging the recorded fluorescence intensity along the optical path to provide a height-averaged concentration field. In order to get the three-dimensional information on the concentration field with high spatial resolution the use of confocal microscopy is indispensable. The major advantage of a confocal laser scanning microscope (CLSM) is the possibility of collecting emitted light only from the focus plane. In front of the detector (photomultiplier) a pinhole is arranged on a plane conjugate to the focal plane of the objective. Light emitted from planes above or below the focal plane is out of focus when it strikes the pinhole. Therefore, most of the light cannot pass through the pinhole and does not lead to the distor-

tion of the image [11]. This spatial filtering is the key principle to increase the optical resolution by producing depthwise optical slicing. The illuminating laser can rapidly scan from point to point on a single focal plane, in a synchronized way with the aperture, in order to complete a full-field image on the detector unit. To reconstruct three-dimensional images the scan is repeated for multiple focal planes under the presumption of stationary conditions [6]. *Yamaguchi* et al. [12] used confocal microscopy to visualize fluorescence intensity patterns in cross sections of a meander channel, allowing a qualitative comparison with CFD results. *Stroock* et al. [13] introduced a confocal microscope to determine the mixing quality in a staggered herringbone mixer at small Reynolds numbers. *Ismagilov* et al. [14] used confocal fluorescent microscopy to visualize the fluorescent product formed by reaction between chemical species in a microchannel. Thus, a quantitative description of reaction–diffusion processes near the walls of a channel allows a profound understanding of three-dimensional hydrodynamics and mass transfer.

The given examples show that several groups have performed investigations into two-dimensional or three-dimensional concentration or velocity fields. Most investigations have been done for passive micromixers at low Reynolds numbers ($\mathrm{Re} < 1$), typical for applications like lab-on-a-chip, microarrays, DNA sequencing or micro-total analysis systems (μ-TAS) in biotechnology.

For chemical reactions, much higher Reynolds numbers are usually necessary (above $\mathrm{Re} > 150$) which lead, even in laminar flows, to the generation of vortices with thin layers of different educts and product concentrations. Even for simple T-shaped junctions, hydrodynamic and mass transfer is a three-dimensional phenomenon that is only describable by quantitative data with high resolution in time and space. A deeper understanding of reactor performance is only achievable with the knowledge of both flow and concentration fields. While the flow velocities and directions give the information about residence time and shear stress, the visualized concentration fields allow the evaluation of diffusion lengths and mixing times. This detailed knowledge will allow the prediction of mixing performance as well as the yield and selectivity of different microfluidic devices in the ambitious field called “chemical microprocess engineering”.

2 Experimental Setup

The experimental setup used for the characterization of microfluidic devices is shown in Fig. 1. The μ-PIV investigations are performed with a conventional epifluorescence microscope (Olympus BX51WI). Deionized water ($T = 20\,^{\circ}\mathrm{C}$) enriched with tracer particles is fed out of pressure containers into the microdevice to prevent any pulsation. The mass flow is measured by mass flow meters FI (Bronkhorst High-Tech BV).

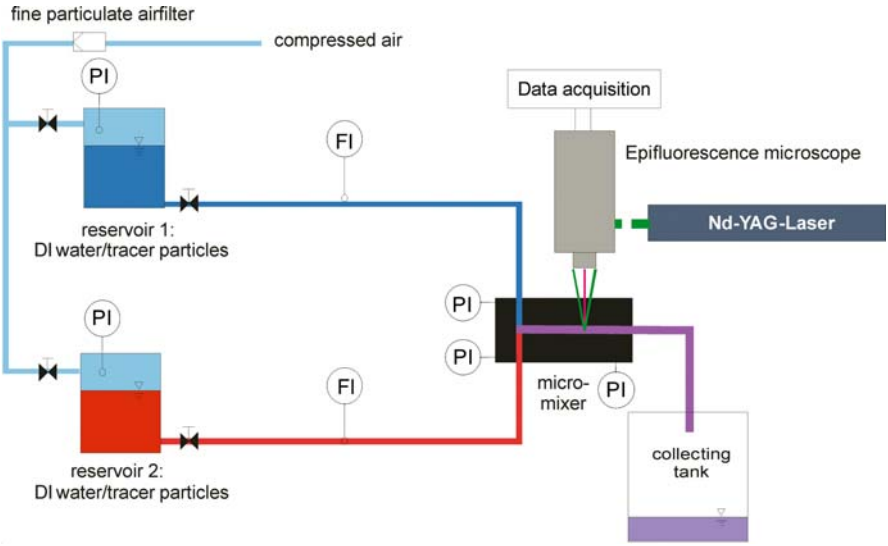

Fig. 1. Experimental setup for flow-field measurements

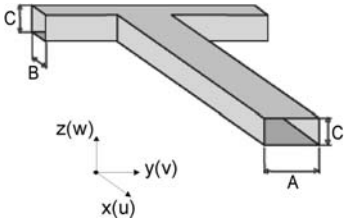

Fig. 2. Geometry of T-shaped micromixer (standard: $A = 200\,\mu\text{m}$, $B = 100\,\mu\text{m}$, $C = 100\,\mu\text{m}$)

The T-mixers with different geometries were provided by the Institute of Microsystem Technology (IMTEK) at the University of Freiburg, Germany [15]. They are made out of silicon sealed by a Pyrex glass lid to allow optical access. The notation for the T-shaped micromixers is $A \times B \times C$ in µm according to Fig. 2.

For µ-PIV nanoparticles with low inertia are used as tracers (polystyrene, particle diameter 500 nm). To detect the invisible particles they are coated with a Rhodamine B layer (Micro Particles GmbH, Berlin) to achieve fluorescence in the laser illumination. Two consecutive laser pulses (wavelength 532 nm; pulse width 5 ns, New Wave Research, Inc.) with a defined pulse distance of 1 µs enable the detection of particle velocities and directions according to the local flow field. A PCO Sensicam QE CCD camera is used

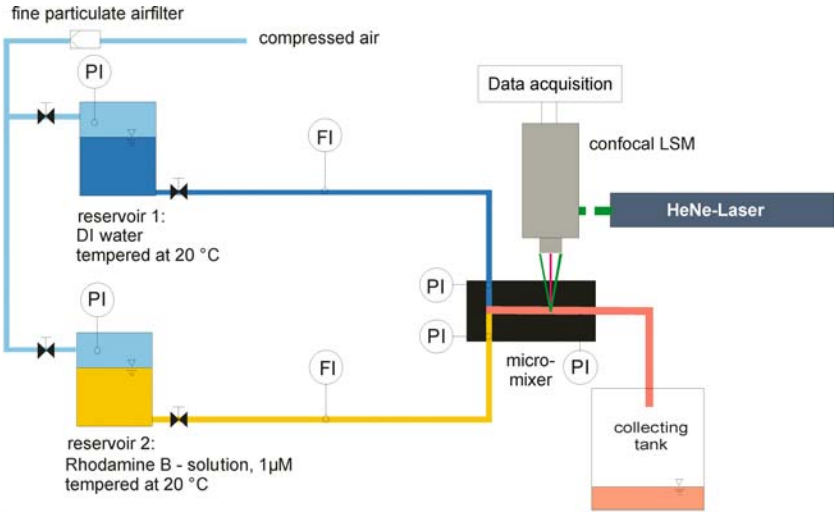

Fig. 3. Experimental setup for concentration-field measurements

for μ-PIV and the data evaluation is performed with a standard crosscorrelation scheme based on a FFT algorithm (μ-PIV system by ILA GmbH, Jülich). The epifluorescence microscope is equipped with a Plan Achromat C Objective (20×/0.4) to achieve a lateral resolution of $7 \times 7\,\mu\text{m}$ with a 32-pixel square interrogation region (half-overlapping) in a measurement depth of approximately $13\,\mu\text{m}$ [16].

For visualization and measurement of local concentration fields a confocal laser scanning microscope (Carl Zeiss LSM 410) is used (Fig. 3). The confocal microscope uses a helium neon laser (543 nm, 1 mW) as excitation source and a 20×/0.5 Plan Neofluar objective. The optical slice thickness is $\approx 8.0\,\mu\text{m}$, the axial resolution $\approx 5.0\,\mu\text{m}$ and the lateral resolution $\approx 0.6\,\mu\text{m}$. A buffer solution (deionized water, pH 8.2 T = 20 °C) is fed out of pressure containers into the microdevice to prevent any pulsation, while the mass flow is measured again by mass flow meters FI (Bronkhorst High-Tech BV) with an accuracy of 1 % of measured value.

One inlet stream is enriched with the fluorescent dye Rhodamine B (dissolved in a pH 8.2 buffer solution) in a very low concentration, thus the fluorescence intensity I_f of the fluorochrome can be assumed as proportional to the concentration of the dye c [16]. Thus, the calibration of grayscales with dye concentrations allows a quantitative analysis of the dye distribution in the microdevice as well as its mixing performance.

For a quantitative analysis of mixing performance Danckwerts' quality of mixing

$$\alpha = 1 - \sqrt{\frac{\sigma_M^2}{\sigma_{\max}^2}} \tag{1}$$

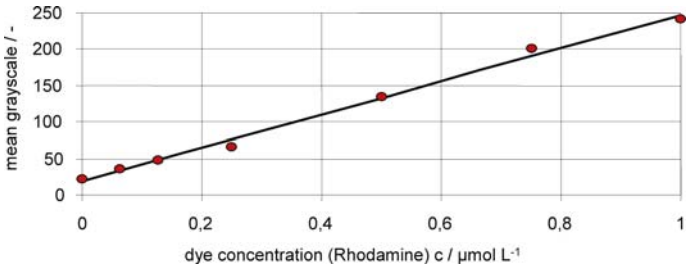

Fig. 4. Grayscale vs. fluorescence dye concentration

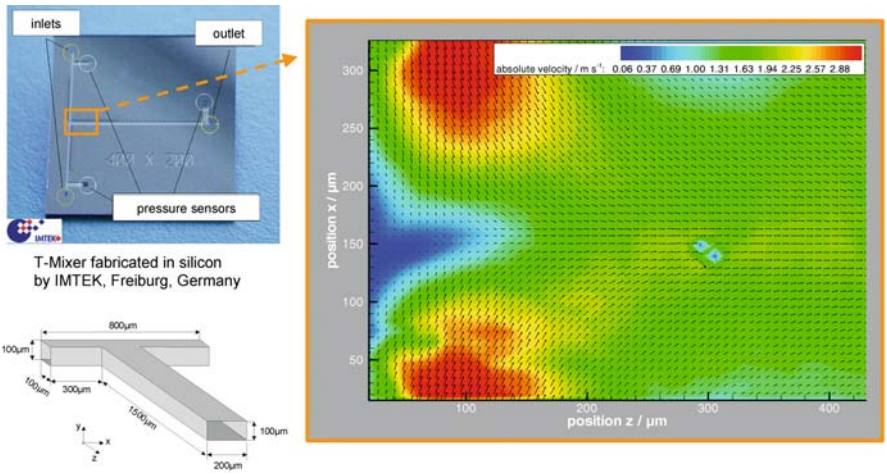

Fig. 5. Plan view of the flow field at the junction of a T-shaped micromixer

can be quantitatively evaluated by means of the standard deviation σ and the maximum standard deviation $\sigma_{\max}$ of concentrations (grayscales) for a cross-sectional area along the channel depth using the Image Processing Toolbox of MATLAB (Mathworks). $\alpha = 0$ corresponds to a totally segregated system, whereas a value of $\alpha = 1$ corresponds to a homogeneous mixture. The image-processing software IMARIS (Bitplane AG, Switzerland) is used to generate a volume-rendered 3D image out of a data set (e.g., 50 slices for a channel 200 µm in depth).

3 Results and Discussion

As stated before, many applications in chemical process engineering as well as biotechnology and analytics require detailed knowledge of the flow field inside microdevices. This becomes obvious in a simple junction that exists in

nearly all microfluidic applications. After draft estimation one would expect a rather good mixing between two fluids in a T-shaped junction. Figure 5 shows the flow field measured by μ-PIV in a T-shaped micromixer edged in silicon by the Department of Microsystems Engineering (IMTEK), Freiburg, Germany.

A wide distribution of velocities between 0.06 m/s at the stagnation point and 2.88 m/s in the deflection zones can clearly be seen. The flow field seems to be very regular and symmetrical along the z-axis. A closer view shows the typical hyperbolic profile as expected by Hagen–Poiseuille's law for laminar flow. This would refer to a very poor mixing due to no cross-sectional exchange of fluid elements. As important parameter for the characterization of the flow regime in microdevices is the Reynolds number Re and is given by

$$\mathrm{Re} = \frac{v \cdot d_\mathrm{h} \cdot \rho}{\eta}\,, \tag{2}$$

with the fluid velocity v, the hydraulic diameter d_h the density ρ and the dynamic viscosity η of the fluid.

A variation of the Re number leads to minor changes in the velocity profiles as shown in Fig. 6 for Re = 120 and Re = 186, although the flow is still laminar. As indicated with the shadow picture in the background of Fig. 6 the flow field is very different between both flow regimes. It has to be pointed out that the asymmetry of the flow is not influenced by differences in the flow rate between both inlets. Even though the measured value of flow rate varies within 1 % independently for both inletes due to the accuracy of the mass flow meters the flow regime is very stable, as indicated by the sharp picture recorded over a period of 8 s.

Furthermore, a good accuracy and reproducibility of the measurements can be shown by comparsion of measured flow rates and integration over the flow fields of 51 layers along the reactor depth. The difference between mass flow meters and PIV measurements is smaller than 1 % with a standard deviation of less than 2 %. These steady flow conditions show a very high reproducablility and thus enable the combination of μ-PIV and CLSM measurements in a sequential mode. The remaining inaccuracy is mainly caused by the difficulties in measuring the high velocity gradients close to the wall. Therefore, the velocity field in Fig. 6 does not fit the wall conditions reliably.

It has to be pointed out that even if the velocity data is important for quantitative calculation and comparison, an illustrative description of the spatial flow field inside a microdevice is not possible.

An illustrative description and quantitative evaluation of the spatial flow field is more effective when using the laser-induced fluorescence as shown in Fig. 7. Combined with the confocal technique that allows detection of emitted light from a discrete plane it is possible to cut the volume of the microdevice into several slices of 7 μm thickness. By using a commercial image-processing software the reconstruction of a 3D image is possible (Fig. 7). An indispensable condition for using the CLSM is the time invariance of the flow field

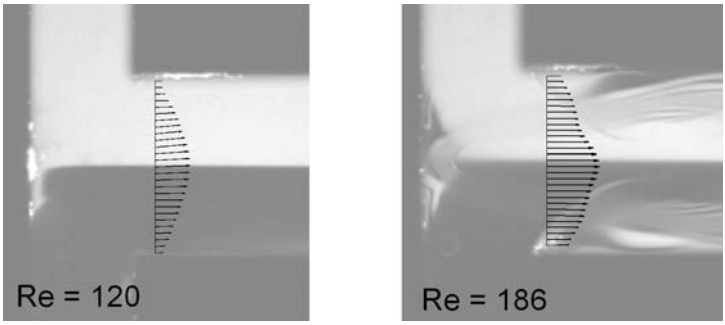

Fig. 6. Velocity profiles at the junction of a T-shaped micromixer for two different flow regimes (measured by μ-PIV)

because the temporal resolution of the scanning technique is in the range of several milliseconds, depending on the spatial resolution. Surprisingly, the flow field in microdevices is stationary and reproducible even though very complex structures occur (Fig. 7, right). The illustrative three-dimensional view into microdevices allows a deep insight into new aspects of mixing. Figure 7 makes it clear that even though only laminar mixing is available in microdevices very fine structures are achievable if the flow regime has been chosen correctly. For example, Fig. 7 shows fine structures with short diffusion lengths down to 3 μm in a T-shaped micromixer. These diffusion lengths are shorter than for almost turbulent flows with large energy input, thus micromixing in microdevices might be more effective than in large facilities. On the other hand, only a reduction of the flow rate to one third causes a tremendous decrease of contact area between two educts and mixing performance (Fig. 7, left).

This can be shown quantitatively with (1) for two T-mixers with different aspect ratio (width mixing channel/height). While the mixing in a device with high aspect ratio is quite poor (Fig. 8, right), an aspect ratio of ≈ 2 enables much higher mixing qualities even for smaller flow velocities (Fig. 8, right). With a higher aspect ratio the development of vortices is supressed and the transition to an engulfment flow occurs at higher velocities. Nevertheless, with one single T-mixer no satisfactory mixing quality is achievable.

Despite the fact that CLSM measurements are helpful for characterizing and designing microdevices and for determining the optimal values for operating parameters, quantitative results for flow velocities, contact times, shear stresses and residence time distributions are only achievable with the help of μ-PIV measurements.

A new method to calculate three-dimensional flow patterns out of two-dimensional PIV data has been recently developed by *Hoffmann* [17]. Hoffmann uses the conservation of mass for incompressible liquids in a closed

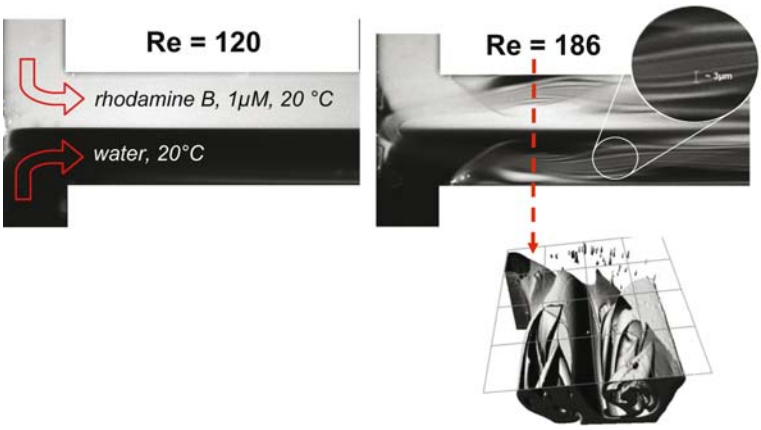

Fig. 7. Plane and spatial distribution of dye in a T-shaped micromixer for two different flow regimes (measured by CLSM)

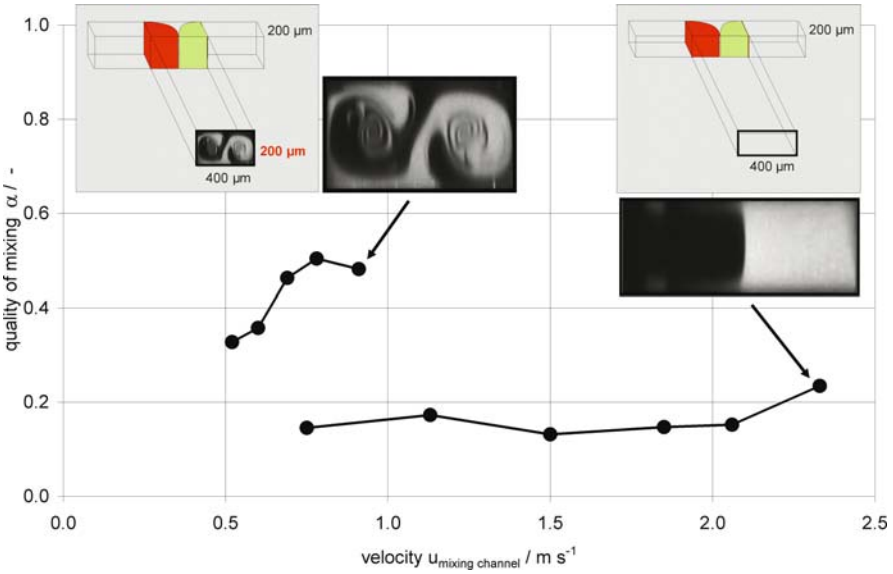

Fig. 8. Quality of mixing α of two mixing devices with different aspect ratio

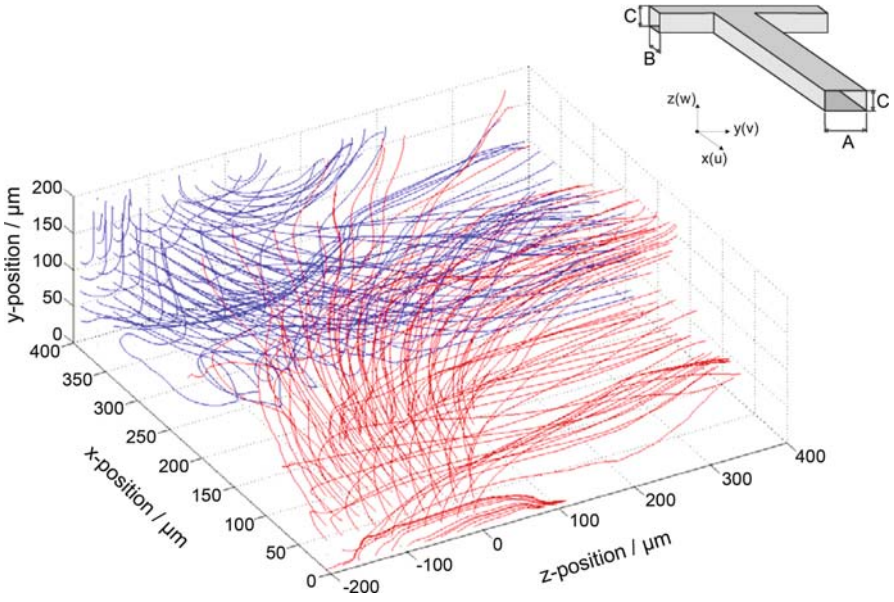

Fig. 9. Velocity streamlines at the junction of a T-shaped micromixer (measured by µ-PIV)

volume with defined inlet and outlet according to the former work of *Feng* et al. [18] for turbulent mixing

$$\frac{\partial u_i}{\partial x_i} = 0 \quad x_i = (x, y, z); \quad u_i = (u, v, w)\,. \tag{3}$$

The missing velocity component w is calculable according to [19] with the continuity equation due to

$$w(x_i, y_j, z_k) = w(x_i, y_j, z_{k-1}) - \int_{z_{k-1}}^{z_k} \left[\frac{\partial u}{\partial x}(x_i, y_j, z) + \frac{\partial v}{\partial y}(x_i, y_j, z) \right] \mathrm{d}z\,. \tag{4}$$

Hoffmann uses a MATLAB algorithm to calculate and evaluate the three-dimensional flow field in micromixers (Fig. 9). The accuracy of the method is evaluated with numerical simulations performed by *Bothe* et al. [20], as well as with three-dimensional measurements carried out with a stereo µ-PIV System by [7].

The knowledge of the three-dimensional flow field allows the calculation of contact times between educts within a microfluidic device as well as residence-time distributions. In combination with the informations about local concentrations of educts and products received from three-

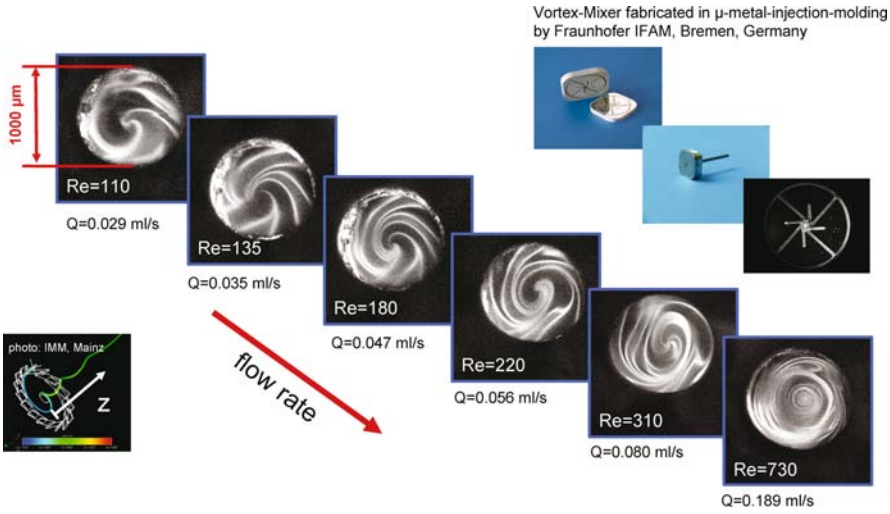

Fig. 10. Plane distribution of dye in a vortex mixer for the synthesis of ionic liquids measured by CLSM [21]

dimensional CLSM measurements, the dependency between the hydrodynamics and chemical/biochemical reactions becomes more apparent and consequently controllable. Therefore, strategies to improve the yield and selectivity will be possible.

The following two examples will show the applicability of the methods for industrial use. For the synthesis of ionic liquids the temperature control of the exothermic reaction is one of the key parameters to ensure a high yield and selectivity. One possibility of achieving a good heat transfer is the use of microreactors with their high surface to volume ratio. A vortex mixer, for example, guarantees a good mixing performance with high heat transfer rates [1]. For the NEMESIS project (BMBF 16SV1970) the Fraunhofer Institute for Manufacturing Technology and Applied Materials Research in Bremen fabricated a vortex mixer out of a metal resistant against most ionic liquids. To check the optimal geometry and flow rate for optimal mixing performance with minimum pressure drop the mixer is fed by water, one inlet stream marked with a fluorescent dye. As shown in Fig. 10 by CLSM, a flow rate of at least 0.08 ml/s should be chosen to achieve a sufficient mixing performance with high contact area between both educts and short diffusion length for micromixing.

Another example concerns the further development of the T geometry by retaining the good mixing conditions in this simple shape. As shown by former investigations [16] it is difficult to achieve a totally mixed flow in a T-shaped micromixer under laminar flow conditions. To ensure a complete mixing in short timescales – which is important for fast chemical reactions

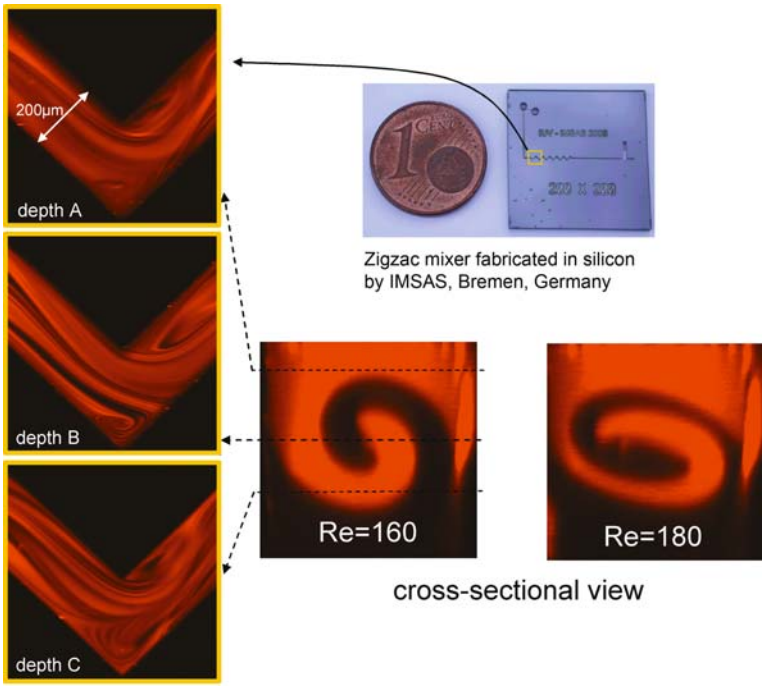

Fig. 11. Plane distribution of dye in a zigzag mixer for parallel consecutive reactions (measured by CLSM)

– a zigzag shape is a reasonable evolution. The zigzag mixer, which is fabricated out of silicon at the Institute for Microsensors, -actuators and -systems, Bremen, Germany (IMSAS) can be characterized by mixing a fluorescent dye into a water stream. Figure 11 shows results of CLSM measurements for three different layers along the depth of the first bend of the mixer as well as the cross section for two different Reynolds numbers. As expected, an increasing formation of vortices becomes apparent with higher Reynolds number, which causes larger contact areas and shorter diffusion lengths with enhanced micromixing.

4 Conclusion

The combined application of µ-PIV and CLSM enables a sophisticated characterization of microdevices. CLSM allows the visualization of flow fields to evaluate flow patterns and mixing performance qualitatively and quantitatively, whereas µ-PIV measurements allow the investigation of velocities, shear stresses, residence-time distributions and contact times. Both techniques together will help to design future generations of microdevices with

a much better adaptation of geometries, materials and surface properties to the demands of reactions than with previous methods. Even if reproducible measurements are possible with the given methods, further development has to be done for enhancement of accuracy. In particular, the measurement of high velocity gradients close to walls and high concentrations gradients inside fine structures are still inaccurate with μ-PIV and μ-LIF. Nevertheless, the extended characterization possibilities will bring a clearer insight into the application range of microdevices for a faster and safer selection of microcomponents. This may guide microprocess engineering to an increased acceptance for the accelerated development of sustainable processes.

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