ABSTRACT

The two dimensional Molecular Tagging Velocimetry (2D-MTV) was used to measure velocity fields of the flow in a micro mixer. Instead of commonly used micro particles an optical tagging of the flow is performed by using a special dye. The flow induces a deformation of the optically written pattern that can be tracked by laser induced fluorescence. The series of raw images gained this way were analyzed and quantified by the “method of the optical flow”. Reference measurements have been carried out to allow conclusions about the accuracy of this procedure.

In addition a quantitative visualization of concentration fields of different fluids that participate in the mixing process of a microfluidic system was enabled by Planar Spontaneous Raman Scattering (PSRS). It was utilized that different species of molecules are distinguishable from another by their characteristic “spectral fingerprints” (their Raman spectra). The Raman stray light of the relevant species was spectrally selected by a narrow bandpass filter and thus detected unaffectedly by the Raman stray light of other species. The successful operation of this measurement procedure in micro flows will be demonstrated exemplary for a mixing process of water and ethanol.

1 INTRODUCTION

Over the past few years micro fluidic systems experienced a rapid development. Technological advancements in the manufacturing processes of micro fluidic components have opened new pathways for a broad variety of technical applications [1,5,8]. Especially chemical and biochemical analysis as well as medical diagnostics, where often only tiny sample amounts are available, strongly benefit from small system volumes and substantially faster analysis times enabled by microfluidic technologies. Recently micro fluidic systems also became interesting for chemical production processes. In particular the possibility to precisely define and control the physical boundary conditions inside micro channels with a huge surface to volume ratio, leads to more efficient production processes with much less by-products.

Due to the strongly increasing interest in micro fluidic devices there is a growing demand for new diagnostic tools for the analysis of flow structures, mixture formation and reaction behavior directly inside the micro channels [7]. In particular non-intrusive measurement techniques which do not influence the flow and reaction processes in the channels are badly needed.

The presented work covers the development of two procedures, which provide comprehensive information about microfluidic flows.
2 FLOW VISUALIZATION

2.1 Flow field analysis by 2D-MTV

For the determination of velocity vector fields of the flow in contrast to approved procedures like PIV or PTV no seeding particles were used as flow markers. Instead the flow was tagged by structured illumination of a fluorescence dye and the pattern written this way was detected time resolved by a camera. The tagging is possible by photo chemical modifications in the dye molecule. Initially the fluorescence ability of the dye is deactivated by an additional functional group added to the molecule, thus this dyes are called “caged dyes” [3]. Applying strong UV light, the chemical bond of this functional group can be broken, so that the original unaltered dye which is able to fluoresce is present again. If the UV excitation is done spatially structured by imaging a mask, a well defined pattern within the dye loaded flow can be generated. The dye can now be excited with a further laser to fluoresce and the spatial fluorescence pattern can be read time resolved by a camera. The image sequences grabbed this way were evaluated by a special algorithm in regard to the flow velocities. The dye diffuses in the fluid and for this reason the written patterns are washed-out in a temporal sequence. Hence classical correlation algorithms as used for PIV are only suitable to a limited extent. Instead a specially adapted version of the “method of the optical flow” (MOF) is used.

Figure 1 shows the experimental setup that was used to acquire the presented results. The object of research (a micro mixer or channel) is located in the center of the setup. The liquid flow inside the micro channels is driven by the gas pressure in a reservoir and controlled by precision valves. The constant gas pressure guarantees a stable and pulsation free flow. A flow meter (SLG 1430, Sensirion) is recording the flow rates synchronously to the image acquisition as reference. The fluid used was demineralized water with a low concentration (500mg/l) of the dissolved “caged dye”. The optical components consist of the part for generating the tagging-pattern in the fluid and a second one for excitation and detection of the fluorescence. For the flow tagging the expanded beam
MICRO-FLOW ANALYSIS BY MOLECULAR TAGGING VELOCIMETRY AND PLANAR RAMAN-SCATTERING

Fig. 2 - 2D-MTV-image series of a laminar micro flow

of a pulsed XeF-Excimer laser (COMPex 150, Lambda Physik AG) at 351nm and a pulse energy of 200mJ is used for a complete illumination of a 40x40mm² mask with a well defined transmission pattern. The image of the illuminated mask is demagnified and imaged into the fluid which flows inside the planar micro mixer (see below). In a second step an Argon-Ion laser (Innova 310, Coherent) at a wavelength of 514nm is used to read out the deformed fluorescence pattern by continuous integral illumination of the dye doped fluid whose fluorescence ability was activated before by the pattern UV-laser beam. A CCD-camera (Imager Compact QE, LaVision) images the fluorescence light of the dye pattern deformed by the flow at well defined points in time after the writing process. Interfering excitation light is suppressed by an optical bandpass filter (OG570, Schott) and an aperture. Further details about this measuring procedure can be found in [6].

Figure 2 shows an image sequence that was generated by the described procedure. This is a typical example for the micro fluidic flows investigated so far. The mixing chamber (5mm x 5mm x 250µm) is streamed from the left to the right in this case. The four side channels that are intended to create different flow fields in the mixer remain unused for these investigations.

Fig. 3 - temporally averaged velocity vector field calculated from the image series in figure 2
A result of the flow analysis with MOF is shown in figure 3. The illustrated vector field of an - in this case - stationary flow is averaged over ten single vector fields, which were calculated from one single image sequence. The resulting vector field reproduces the anticipated flow field very well. More details about MOF can be found in [2,4,6].

2.2 Reference measurements and Taylor dispersion

A disturbing effect which is particularly distinct in micro flows is the so called Taylor dispersion. It is caused by the parabolic velocity profile that forms between top and bottom plates in a flat channel. By means of this effect the fluorescing patterns blur increasingly during the progressing flow. This issue is drafted in figure 4. This kind of detection, which integrates over the depth of the channel, leads to the deformation of the initially punctual pattern to a kind of “comet tail” that can be found for example in figure 2. The evaluation results in lower velocities for the region of the “tail” and higher ones for the front of the moving pattern. Due to the fact that the velocity values in the vector field need to be allocated to different levels in the channel the underlying Taylor dispersion makes conclusions about the accuracy of the procedure difficult at this state of the work. The mean value averaged over the blurred pattern however is in relative good agreement with the preset values but the local variations may be in high gear.

![Fig. 4 - schematic diagram of the pressure driven flow between top and ground plate](image)

At present methods are investigated for the extraction of the velocity vector field in the middle level (apex of the Taylor dispersion parable) from the raw data images which were integrated over the channel depth.

The present state of the work does not allow selecting single levels in the channel. For a comparison of the vector fields that were evaluated by MOF but that could not be corrected concerning the Taylor dispersion with a reference value yet, measurements in a straight channel with a rectangular profile of 5mm x 250µm were carried out. From data simultaneously gathered with a flow meter it was possible to calculate mean flow velocities in this simple channel very precisely. The mean flow velocity was also calculated from the measured velocity vector fields by a local average determination. Figures 5a and b show the comparison of the temporal sequence of both mean velocities. The measurements differ in the mask used for the tagging and the preadjusted flow velocities. For figure 5a a mask with a periodical dot pattern was used, where the dots had a diameter of 320µm and are 960µm apart from another. The mask for the measurements shown in figure 5b contained dots of only 150µm diameter and 225µm distance. Additionally the flow velocities adjusted for the measurements for figure 5b were twice as high as for the measurements for figure 5a. Thus the tail like distorted patterns are flowing into another very fast. In contrast to this the patterns of the measurements for figure 5b are flowing into each other much later and very slowly. If
this happens the basic assumptions for the functioning of MOF are violated. This leads to flow velocities that are too high. In Figure 5b this tendency becomes apparent imposingly while figure 5a shows a measurement that is in relative good accordance with the reference value measured by the flow meter.

3 DETERMINATION OF SPECIES CONCENTRATIONS BY PLANAR SPONTANEOUS RAMAN SCATTERING

The determination of concentration fields in micro mixers is carried out by a second procedure. The measuring procedure uses the fact that different molecular species are clearly distinguishable from each other by means of their characteristic Raman spectra. In this “spectral fingerprint” it is normally possible to find identifying features for the differentiation in the form of particular Raman bands that are characteristic for a single species. Narrow bandpass filters allow the spectral separation of the Raman stray light of the relevant band without or with only minor interferences from other species. The local Raman stray light intensities obtained this way are a direct measure of the density distributions of the examined species.

The first tests were done with water and ethanol as the sample, since they are easily available
and are easy to handle. Furthermore ethanol is an organic solvent and hence has an intense characteristic band in the Raman spectrum at approx. 3000cm\(^{-1}\) (CH-band). The Raman stray light of this band is selected with the help of a proper narrowband filter. Figure 6 shows an overlay of the Raman spectrum of ethanol with the transmission curve of the used filter.

![Fig. 7 - oversight sketch of the experimental setup for the two dimensional determination of concentration fields by planar Raman scattering](image)

The experimental setup is drafted in figure 7. At first a cuvette of fused silica (type 23, Starna) was used as a flow cell. It was filled with either a well defined concentration of the two substances or pure demineralized water into which the ethanol was injected by a syringe. The excitation was done by a pulsed Nd:YAG laser at 532nm (Brilliant B, Quantel). The intensity of the Raman stray light strongly depends on the excitation wavelength. Due to this strong dependency \((I_R \sim 1/\lambda^4)\) ideally a laser with a short wavelength should be used in order to gain strong signals. Attempts with a UV laser however resulted in unsolvable problems because of strongly superimposed fluorescence emissions. Hence visible light at 532nm was used for the excitation.

The pulsed, frequency doubled Nd:YAG laser beam is formed to a small light sheet (approx. 800\(\mu\)m thick and 12mm high) by a lens system. The light sheet selects a plane in the cuvette that is imaged with an image intensified camera (Flamestar III, LaVision) through the Raman filter (633FS10-25, LOT Oriel). The Raman filter represents the central element for these examinations. It has to be precisely fitted to the Raman spectra of the examinated substances and should feature a transmission as high as possible.

At first the cuvette was filled with pure demineralized water. The observation through the Raman filter that selects the fundamental CH-stretching oscillation of the hydrocarbons (approx. 3000cm\(^{-1}\)) makes sure that almost no light reaches the camera. However the characteristic OH-oscillation Raman band of water slightly overlaps with the transmission band of the filter in such a way that minor parts of water are detected too. To generate a first scenario of instationary mixing conditions of ethanol and water, ethanol was injected from the bottom into the cuvette filled with pure water. The Raman stray light of the CH-band of the alcohol passes the Raman filter and is detected by the camera. Initially the ethanol ascends to the top because of its lower density as apparent in figure 8a-f. The figures show consecutive snapshots of the ethanol distribution during the progressing injection. For recording the images only a single laser pulse with 30mJ pulse energy and 5ns pulse duration was used. These images demonstrate that selective imaging of single molecular species in a mixture is feasible. However problems arise from different refraction indices of ethanol.
and water, which lead to beam steering effects resulting in strongly increasing inhomogeneities in the laser light sheet during the progressing mixing process. These beam inhomogeneities clearly appear in form of streak patterns in figure 8. In addition to the different refraction indices of alcohol and water both substances noticeably differ in their specific densities, too. Hence mixing processes of these two substances will always be influenced by gravitation, which leads to unintentional convections.

The intensity data has to be converted to concentration values since the procedure should enable a quantitative mapping of the concentration values of a species. For calibration purposes both substances have been homogeniously mixed in the cuvette in well defined mass fractions and are then imaged by the Raman system. The detected Raman stray light intensities are directly proportional to the density of molecules in the measurement volume, which again is proportional to the mass of all molecules of the selected species. The fraction of ethanol was calculated by normalization with the known maximum value from the image of pure ethanol. Figure 9 shows the
measured ethanol mass fraction compared to the preset mass fractions of ethanol. The anticipated ideal value is plotted in form of the bisecting line. The measured values are very close to the ideal ones but show systematic discrepancies to higher values. The reason for these discrepancies is not known right now and matter of current work. It is assumed that there is a correlation to the volume contraction which occurs during mixing of molecules with different size.

4 CONCLUSION

Two dimensional Molecular Tagging Velocimetry was successfully used to determine velocity vector fields in two dimensional micro flows. The calculated values are in good accordance to reference measurements. Obvious discrepancies are resulting under certain conditions due to the influence of Taylor dispersion. Appropriate corrections have to be implemented in order to obtain more precise velocity values. Currently different approaches for this correction are implemented into the evaluation algorithm.

If the fluorescence patterns are flowing into another a systematic discrepancy between preset velocity and flow velocity measured by the tagging method occurs because the assumptions for the analysis by the “method of the optical flow” are violated. A trade off between pattern density and observation time has to be made to avoid this. For a further improved reference comparative μPIV-measurements are underway. After a correction of the discrepancies due to Taylor dispersion this measurements should allow a direct comparison of the resulting two dimensional vector fields.

The procedure of planar spontaneous Raman scattering was successfully tested on simple model systems. The first visualizations of concentration fields of an ethanol flow in water show the potential of this technique. Evaluations of the linear correlation between recorded stray light intensities and preset concentration value resulted in good accordance.

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