Micro-Particle Image Velocimetry (μPIV): Recent developments, applications, and guidelines†

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In this review we discuss the state of the art of the optical whole-field velocity measurement technique micro-scale Particle Image Velocimetry (μPIV). μPIV is a useful tool for fundamental research of microfluidics as well as for the detailed characterization and optimization of microfluidic applications in life science, lab-on-a-chip, biomedical research, micro chemical engineering, analytical chemistry and other related fields of research. An in depth description of the μPIV method is presented and compared to other flow visualization and measurement methods. An overview of the most relevant applications is given on the topics of near-wall flow, electrokinetic flow, biological flow, mixing, two-phase flow, turbulence transition and complex fluid dynamic problems. Current trends and applications are critically reviewed. Guidelines for the implementation and application are also discussed.

Introduction

With more advanced and more complex microfluidic devices the need grows for a detailed investigation of the fluid mechanics in such systems. Fluid mechanics at small scales can be investigated analytically, numerically by Computational Fluid Mechanics (CFD) or experimentally. The most elemental method of experimental investigation is flow visualization. The fundamental idea of flow visualization is to alter the working fluid in a way that the fluid motion stays unchanged while the fluid transport is made detectable. A typical example is given by Seger et al. Here the flow of cells is made visible by using bright field illumination, where objects with a lower light transmission appear as dark spots. Simultaneously the flow of the continuous water phase is made visible by partly seeding the working fluid with a scalar marker, a fluorescent dye at a different light wavelength (Fig. 1). With the use of optical filters in the beam path of a microscope the fluorescent fluid can be discriminated from unseeded fluid. A similar approach is used by Stroock et al.

Fig. 1 Example of flow visualization. The flow of cells is made visible by using bright field illumination, where objects with a lower light transmission appear as dark spots. Simultaneously the flow of the continuous water phase is made visible by partly seeding the working fluid with a scalar marker, a fluorescent dye at a different wavelength of light (from Seger et al. – Reproduced by permission of The Royal Society of Chemistry).

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to visualize the 3D-flow structure in herringbone-type mixer device by means of confocal laser scanning microscopy. Other methods of flow visualization are based on phosphorescence, photo-bleaching, molecular tagging of a caged fluorescein and Raman-scattering. A review of microscale visualization methods is given by Sinton. While the results obtained with the methods mentioned above are qualitative, other methods generate quantitative data. These methods are called quantitative flow visualization. Scalar-based visualization methods can be made quantitative by image processing of the scalar distributions. An alternative way is to use discrete particle distributions instead of a scalar marker to visualize the flow. The most established method of this kind is digital particle image velocimetry (PIV). PIV is an optical, non-intrusive whole-field method. Particle distributions are recorded at two instants of time and from the change of the particle distribution over time, the flow motion is determined. In the following chapter the method will be discussed in detail. While several biomedical researchers applied micro-scale particle tracking (PTV) before, in 1998 Santiago et al. introduced micro-scale Particle Image Velocimetry (μPIV) where the velocity field is calculated by cross-correlating the acquired images. In the last 10 years this method has become the standard tool for fluid velocity measurements in microchannels.

The advantages of the μPIV-method are manifold. First of all μPIV is in a well-developed state and turn-key systems can be purchased from various suppliers. From the end-users point of view the output format of the velocity data as a vector plot, where individual vectors indicate the magnitude and direction of the velocity at the local position has advantages over other methods that generate e.g. line plots. The vector plot format is intuitive and it allows a direct comparison with CFD data, which is part of the reason for the acceptance of the method in industry and application-oriented research. With μPIV two-dimensional velocity fields are obtained in one two-dimensional plane with finite thickness in the flow. The data is obtained optically, illumination and recording of the flow are performed through the same microscope lens, thus no changes in the MEMS design (e.g. no electrodes, no light guides) are necessary except optical access from one direction. The method is non-intrusive and the amount of energy added by the illumination is small, so the fluid flow is not altered by the measurement. The method is very flexible; regions from a few micrometers up to several millimeters can be investigated by choosing an appropriate magnification. Flow velocities from nanometers per second to meters per second can be measured by adapting the timing of the measurement devices to the flow problem. Extensions of the method exist for specific problems, like three-dimensional velocimetry with a stereo-microscope or near-wall measurement in the region 100 nanometers away from the wall, although those methods are delicate to apply and are recommended for the experienced μPIV-user.

In this paper we give a critical review of the micro-scale Particle Image Velocimetry (μPIV) method. In chapter 2, the method is introduced, in the following chapter practical guidelines and considerations are given. We introduce a number of applications and interesting papers on various topics without the intention of completeness. The paper ends with a summary and critical conclusion.

**Description of μPIV**

**Measurement principle**

The measurement principle is based on Particle Image Velocimetry for large scale applications. The optical velocimetry technique uses conventional microscopy and digital imaging methods for the quantitative determination of two-component velocity data in a two-dimensional measurement plane. Depending on the choice of microscope objectives regions of investigation range from 1 × 1 mm² for a 5x magnification lens to 50 × 50 μm² for a 100x magnification lens. Within the region

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of interest velocity information spacing is of order 1 μm. Spatial resolution up to a few 100 nm can be achieved.

For the optical measurement technique a transparent working medium and optical access to the area of investigation is needed. The flow is seeded with tracer particles for the visualization of the motion of the fluid. Ideally the tracer particles should follow the flow. This is achieved by matching the density of the fluid and of the flow-tracing particle or by the use of very small particles. On the other hand Brownian diffusion is significant for particle diameters smaller than 1 μm and introduces a significant error in the velocity determination.19 The error by Brownian motion can be eliminated using correlation averaging in the PIV evaluation as described below. For microfluidic applications with water as a working fluid the tracer particles are typically made of polystyrene and have diameters of 200 nm to 2 μm. Pouya et al.20 introduced quantum dots (QD) as tracer particles. With a diameter of 5–25 nm quantum dots might become a very useful tracer for nano-scale velocimetry. Their emission wavelength can be tuned and surface coatings for solubility in organic or inorganic solvents can be controlled.21,22 Disadvantages of QD as flow tracers are their random blinking behavior, their large Brownian motion and the weak intensity signal. Freudenthal et al.23 used tracer particles of 70 nm diameter with QD conjugated on the particle surface. With this approach the influence of blinking is removed and the signal intensity is increased at the cost of a larger particle.

A volume of liquid with tracer particles is illuminated and observed with the aid of a microscope objective. For volume illumination all tracer particles emit light, but only the light originating from tracer particles in the focus plane of the microscope objective (and of those slightly out of focus) is originating from tracer particles in the focus plane of the observed with the aid of a microscope objective. For volume particle surface. With this approach the influence of blinking is eliminated using correlation averaging in the PIV evaluation.

A typical PIV realization consists of a pulsed light source that is synchronized with the digital camera by a timing unit. The synchronization is done in a way that the first light pulse is set at the end of the first camera recording and the second pulse is set at an arbitrary time in the second recording. In this way the time interval Δt is independent of the camera frame rate, but defined by the time interval between the two synchronized light pulses. The time interval can now be adjusted to the flow conditions.

Frequency doubled Nd:YAG lasers at 532 nm wavelength of the laser light are a standard pulsed light source for PIV. For PIV applications less than 10 mJ of laser power per pulse is sufficient. Double cavity lasers consist of two lasers that can be triggered independently and of a beam-combining optic for the adjustment of the overlap of the two laser beams. Frequency-doubled Nd:YAG lasers are robust, have a good beam quality and the light can easily be coupled into the standard beam path of a microscope. Alternative light sources are diode-pumped lasers like Nd:YLF lasers that have a longer pulse length. This increases the signal intensity of fluorescent tracers. Ongoing development of laser diodes and high-power LEDs will increase the applicability of these types of light sources when used to illuminate PIV recordings. The current limitation using LEDs for fluorescence imaging is that a longer illumination pulse than that of a laser is required in order to achieve the same fluorescence signal intensity. For that reason LEDs are not suited for measurements of moderate or fast flows.24

For a high image quality the camera should have the highest possible sensitivity/quantum efficiency. If the signal quality is insufficient intensified CCD cameras are an alternative but the image quality of intensifiers is poor. The use of double-shutter cameras reduces the time interval between two consecutive recordings to 1 μs allowing measurements of high velocities at high magnification.

The image formation of a μPIV recording is typically dominated by large magnification and high numerical aperture (NA) of the microscope objective. As a consequence the influence of diffraction-limited optics is reduced and the image formation is dominated by geometric optics. Ooms et al.179 developed a model to estimate the effective numerical aperture of a microfluidic optical experiment.

The quality of a μPIV recording is significantly improved by fluorescence imaging.26 Fluorescent tracer particles are added to the flow. Fluorescence is exited by illumination with monochrome laser light and the tracer emit light shifted to a longer wavelength, while all other obstacles like the walls of the microchannel reflect and scatter light at the original wavelength. An optical filter in the microscope is placed between the light collecting objective and the digital camera. The optical filter reflects light at the illumination wavelength and transmits the fluorescent light that has a longer wavelength. With this optical setup, light originating from the fluorescent tracer particles is collected on the camera sensor, while light originating from the channel walls and other non-fluorescent disturbances is blocked by the optical filter. This significantly improves the signal to noise ratio of the μPIV measurement. A schematic of a μPIV set-up is shown in Fig. 2.
The PIV measurement data are evaluated by means of cross-correlation. For the evaluation the digital images are divided in a uniform grid of so-called interrogation windows (IWs). The size of the IW is chosen small enough so that the particle displacement in the region is uniform, while on the other hand the IW should be large enough so that it contains sufficient information for the evaluation. The image patterns in the IWs in the images recorded at $t_1$ and $t_2$ are compared statistically, i.e. a spatial correlation analysis is performed. For a certain relative shift $\Delta s$ of the two evaluation windows in the first and second image, the recorded image patterns match, i.e. they exhibit a high correlation. The procedure is repeated for all IWs resulting in a uniform grid of displacement information.
With an image of an object of known length (e.g., a calibration target or the microchannel width) one can determine the exact magnification $M$ of the imaging system. The product of the displacement of the particle image pattern $\Delta s$ and the magnification $M$ is the displacement of the particles in the object plane $\Delta s$. With the known time interval $\Delta t$ the particle velocity over one interrogation region can be calculated as follows $v = M(\Delta s/\Delta t)$.

The cross correlation evaluation for all IWs yields a uniform grid of the two in-plane components of the velocity vector in a two-dimensional measurement plane, which is typically represented by a vector field.

We remind that the two-dimensional measurement plane has a certain thickness which is determined by the depth of field of the objective lens as explained above. This thickness of the measurement volume is an important experimental parameter in PIV and is typically expressed in terms of the depth of correlation, i.e. the depth over which particles significantly contribute to the correlation. The correct quantification of the depth of correlation, especially under different operating conditions, is still the object of current investigation. However, it is commonly accepted to use the following simplified analytical expression derived by Olsen and Adrian and Bourdon et al. to quantify the depth of correlation (DOC) for a standard μPIV setup:

$$\delta_{DOC} = \frac{2}{\sqrt{\epsilon}} \left( \frac{n_0 d_p^2}{4 N A^2} + \frac{5.95(M + 1)^2 \lambda^2 n_0^2}{16 M^2 N A^4} \right)^{1/2}$$

where $d_p$ is the particle diameter, $\lambda$ the light wavelength, $M$ the image magnification, $n_0$ is the refractive index of the lens immersion liquid, $N A$ the numerical aperture of the lens, and $f$ the f-number of the optical lens. The first-order approximation $N A = n_0/2f$ has been used with $f$ being the f-number of the optical lens. $\epsilon$ is the relative threshold below which the defocused particle images no longer contribute significantly to the displacement-correlation peak, normally set to be equal to 0.01. From equation (2) it is evident that $N A$, $d_p$, dominantly contribute to the resulting depth of correlation, the magnification $M$ has less influence. For example, using a $M = 40$ lens with a typical $N A = 0.75$ yields a DOC of 4.0 μm for particles of approximately 200 nm and a DOC of 12.6 μm for 3.0 μm particles. For a constant particle diameter of 1.0 μm, the DOC increases from 2.4 μm to almost 39.5 μm if an $M = 60/N A = 1.4$ or $M = 10/N A = 0.25$ lens are used, respectively. The concept of depth of correlation is schematically illustrated in Fig. 3.

Very often μPIV experiments suffer from a very low particle image density (the number of particle images in one interrogation window) due to the small depth of the measurement volume for high numerical aperture (NA) microscope objectives. For stationary flows (or pulsating flows with a phase-locked data acquisition) correlation averaging during the cross-correlation evaluation is a powerful method to increase the number of particle images contributing to a μPIV evaluation. Here, the correlation matrices of several measurements are summed up and the peak in the correlation (that represents the matching particle image pattern) is searched in the sum of the correlations. This procedure results in increased evaluation fidelity (Fig. 4). The analysis of the efficiency of correlation averaging in Fig. 5 shows that under typical experimental conditions 50 image pairs are sufficient to obtain close to 100% valid vectors. This value can increase for unfavorable experimental conditions (low particle concentration, high noise level). Correlation averaging can also be used to increase the spatial resolution of a μPIV measurement. The acquisition of two-dimensional data scanned through the out-of-plane direction can be used to reconstruct a three-dimensional flow field.

**Instruction for users**

The implementation of μPIV experiments, even those conceptually simpler, goes often along with unexpected troubles and
frustrating, time-consuming practical problems. Impossibility to get clear particle images, difficulties in achieving a stable flow rate in the device, meaningless velocity vector fields obtained from the evaluated data, are annoying occurrences frequently encountered by μPIV users, either with or without experience on the field.

In this section we present and discuss practical advise based on experience that we hope can be useful to build up a successful μPIV experiment.

**Illumination**
- Laser light is coherent, which results in speckle (multiple interference of coherent light) that disturbs the image quality. The speckle can be removed by putting a holographic light-shaping diffuser plate in the beam path as shown in Fig. 2.
- Use a plastic holographic diffuser plate (price about US$100). When the laser power is accidentally set too high the plate will burn and become opaque. The opaque plate protects expensive optics from laser damage.
- Be careful that focal points are not present in the light beam forming optics. Even at low power, the laser light focused into a lens can severely damage the glass.
- When using fluorescence imaging remember that the intensity of the light emitted by the fluorescent particles (and collected by the digital camera) increases proportionally with the excitation light intensity only until a saturation level. Increasing further the illumination intensity beyond that level will not further improve the signal intensity, but will result in an increase of the background noise and in the bleaching of the fluorescent dye, i.e. decrease of the signal to noise ratio.

**Seeding**
- Try to avoid high tracer particle concentration. If possible consider using correlation averaging techniques to virtually increase the particle image density. High particle concentration brings undesired problems such as higher background noise or particle agglomerations.
- The choice of the tracer particles size is a crucial issue in μPIV. The size strongly affects the error of the measurement. Numerous partially contradictory fluid-mechanical, optical and evaluation-based requirements need to be respected such that often only a compromise solution for the particle size can be found. In the following the most general issues will be discussed:
  - Particles must be significantly smaller (at least two order of magnitude) than the relevant fluid dynamic length scale, i.e. the channel diameter. So no size effects are expected.
  - Particles need to follow the flow, that is, particles with a sufficiently high response time \( \tau_p \) need to be chosen. The particle’s behavior under acceleration is given by
    \[
    \tau_p = \frac{\rho_p}{\rho_f} \frac{d_p^2}{180v_f}\n    \] (3)
    where \( \rho_p \) is the particle density \( \rho_f \) is the fluid density and \( v \) its kinematic viscosity. This response time should be smaller than the smallest time-scales in the flow.
- Brownian motion limits the minimum particle size. Santiago et al. gives the error due to Brownian motion relative to the mean in-plane displacement by
  \[
  \varepsilon = \left( \frac{s^2}{\Delta x} \right)^{1/2} = \frac{1}{n} \sqrt{\frac{2D}{\Delta t}} = \sqrt{\frac{2}{\Delta t}} \frac{k_b T}{3 \pi \mu d_p} \] (4)
  Herein, \( D \) is the diffusion coefficient given by the Stokes-Einstein relation, \( k_b \) Boltzmann’s constant, \( T \) is the temperature in Kelvin, \( \mu \) the fluid’s dynamic viscosity, \( \langle s^2 \rangle \) is the rms of typical fluctuations due to Brownian motion, \( \Delta x \) is a typical particle displacement between two consecutive recordings, which can be expressed by the particle velocity \( u \) and the time interval \( \Delta t \). For a 1 μm diameter particle the diffusion coefficient is typically \( D \sim 10^{-12} \) m²/s. Especially, for slow flows when \( \Delta t \) is large, e.g. 33 ms, the error in the diffusion length, i.e. the error in the displacement measurement exceeds 100 nm. Note that the diffusion length of
Brownian motion in the vicinity of walls tends to be non-gaussian distributed.

- The imaged particle diameter $d_e$ on the camera chip is a combination of the physical particle diameter $d_p$ and of the diffraction size $d_d$. Following Olsen and Adrian,\textsuperscript{32} the particle image diameter is given by

$$d_e = \left[ M^2 d_p^2 + 5.95 \cdot (M + 1)^2 \lambda^2 \left( \frac{n_0}{\Sigma N} \right)^2 \right]^{1/2}$$ \hspace{1cm} (5)

From this relation it becomes clear that the diffraction term is independent of $d_p$ and becomes dominant at a given $M$ and $N\lambda$ if the particle diameter decreases. That is, optical diffraction limits the minimum achievable resolution of a µPIV system. Following Raffel et al.,\textsuperscript{40} the particle image on the sensor $d_e$ should be in the order of 2–3 pixels.

- Particle visibility changes with particle-image diameter. While for particles larger than the wavelength of the scattered light the intensity is proportional to the $d_p^2$, for particles smaller than the wavelength, the intensity varies with $d_p^m$, where $m = 4...6$.\textsuperscript{41,42} That is, the intensity decreases extremely fast with decreasing diameter.

- The random error amplitude $\sigma_{\Delta \chi}$ in the measured displacement is approximately proportional to the diameter $d_p$ of the displacement correlation peak:\textsuperscript{41,43}

$$\sigma_{\Delta \chi} \approx \epsilon \frac{d_p}{\sqrt{2}}$$ \hspace{1cm} (6)

where $\epsilon$ is a constant related to the experimental parameters.\textsuperscript{43} For a uniform displacement of the tracer particles in the interrogation domain the diameter of the correlation peak is proportional to the particle-image diameter $d_p = \sqrt{2}d_e$. This expression is exact when the particle image shape is Gaussian. The value of $\epsilon$ is typically around 0.05–0.10.\textsuperscript{41,43,44} If the flow is non-uniform, i.e., if shear gradients are present, the random error will further increase due to a broadening of the correlation peak diameter $d_p$.\textsuperscript{45}

- Recommended particle size for standard microfluidic devices is 500–1500 nm, which offers a good compromise between visibility and flow fidelity.

- Use monodisperse tracer particles for uniform particle image intensity.

- For experiments in water the use of hydrophilic coating for tracer particles significantly reduces adhesion of particles to the wall. Particles with polyethylene glycol (PEG) coating are commercially available for best results. Remember that the quality of the coating ages.

- Rinse and clean the microchannel as best as possible at the end of each experiment. An ultrasound bath can help to remove tracer particles attached to the walls.

- Tracer particles carry surface charge. In an electric field they will not behave neutral, but they will migrate by electrophoretic or di-electrophoretic forces.\textsuperscript{46}

**Optics**

- To avoid strong distortions adopt optical access with planar surfaces at the interfaces. When curved surfaces cannot be avoided use refractive index (RI) matching.\textsuperscript{47} For instance there exists transparent TEFOLON that has almost the same refractive index as water and can be used to produce round capillaries.

- The optical filters in the microscope have to be chosen with respect to the emission wavelength of the dye. On the detection side the excitation wavelength needs to be cut off completely. It is advisable to use high quality optical filters with high transmission in the emission wavelength range.

- Carefully select the objective lens for the experiment:
  - Use a lens with a high numerical aperture (NA) and planar correction. High quality objectives are the most cost-efficient way to improve the image quality.
  - Immersion lenses (oil/water) can be used to achieve a higher NA and a smaller depth of correlation but typically have small working distances (WD).
  - When the optical access is a glass coverslip check that the objective lens has a suitable correction for the corresponding coverslip thickness.
  - Check that the WD of the objective lens is large enough for the application. Remember that the actual WD is different from the WD reported in the lens specification when the optical path travels through media with different RI.
  - µPIV works best for penetration depth of up to 200 µm. For larger distances the blur of defocused particles dominates. This error source can be reduced by decreasing the particle concentration.

**General remarks**

- The use of an inverted microscope system is advisable in most cases. Inverted microscopes are more rigid and stable and provide more space for the set-up where usually room for tubing and other devices is needed. For certain experimental conditions, e.g., in an open Petri dish when a water dipping lens is needed or when sedimentation of heavier objects like cells block the view through the bottom of the microchannel, an upright microscope is an alternative.

- Generate a stable flow before running the experiment. Fluctuations and instabilities in the flow rate might not be apparent in time-averaged µPIV measurement but lead to an erroneous estimate of the actual velocity field. Practical advice is:
  - Avoid air intrusions in the microfluidic devices. The formation of bubbles, when not expected, is usually an indication of leakage or faulty operation of the device. Check the sealing of the tubing. Degassing the working fluid in a vacuum chamber can help to reduce bubble formation.
  - An increase of the pressure drop, for instance using longer tubing, can be useful to stabilize the flow.
  - Use little compliance tubing to prevent a drift in the pressure and flow rate due to expansion of the tubing.
  - Use pulsation reduced syringe pumps, e.g., a pump that is driven with a servo drive instead of a stepper motor. If possible use hydrostatic pressure to drive the flow, which is pulsation free.
  - Record time series of velocity data to check for unwanted variation in the flow rate. Although the accuracy of the measurement will be rather low, it will be sufficient to check if not-negligible random or periodic fluctuations are present in the flow.
  - Only when the RI of the immersion fluid is identical to that of the working/measurement fluid, the translation of the objective lens directly corresponds to the shift of the focal plane. Otherwise, the lens translation needs to be calculated with respect to the different RIs of the fluids.
• Set a correct exposure time interval $\Delta t$ between the image pair:
  - A long $\Delta t$ reduces the influence of the absolute error.  
  Use long $\Delta t$ when the flow is two-dimensional and strong velocity gradients are not present.
  - Reduce $\Delta t$ in case of large out-of-plane motion or strong velocity gradients to reduce the relative error due to de-correlation. On the other hand, from equation (4) it also becomes evident that for short $\Delta t$ the relative error due to Brownian motion becomes larger.\(^{17}\)

### Applications

The following section will give an extensive but not complete overview on publications in the literature which report on the use of $\mu$PIV in micro-fluidic devices. Some of the papers only report on the use of “standard” $\mu$PIV applied to measure the 2D flow field at the centerline of simple geometries, whereas others describe the development of partially very sophisticated $\mu$PIV instrumentation to study the flow in more complex geometries and in more depth. The range of possible applications is extremely wide and the focus can be rather a technological one (e.g. optimization of micro-fluidic devices) or the investigations can be of fundamental fluid-mechanical interest. Some of the points (e.g. transition to turbulence on a micro-scale) are under discussion in the literature.

For each of the following fields of application, a short introduction will shortly discuss further specific difficulties and pitfalls related with the measurements.

#### Near-wall flow

Velocity measurements in near-wall or interfacial regions are often needed in microfluidic applications, especially as a consequence of the high surface/volume ratio. Near-wall regions are often characterized by strong velocity gradients that can cause shear-induced migration of particles\(^{24}\) and reduce the accuracy of $\mu$PIV measurements.\(^{45,48}\) When the wall coincides with the optical access, a reduction in visibility can occur especially in long run experiments, due to tracer particles agglomeration and adhesion to the wall.

On the other hand near-wall $\mu$PIV measurements can be extremely useful to characterize quantities related to the fluid-solid structure interaction. For example 2D $\mu$PIV measurements perpendicular to a wall in a microchannel can be used to determine non-invasively the shapes of the wall with resolution approaching tens of nanometers as shown by Stone \textit{et al.}\(^{49}\) Assuming the no-slip boundary condition, the wall position is extrapolated from the measured velocity profiles. The same principle was used by Rossi \textit{et al.}\(^{46}\) to reconstruct the topography and shear stress distribution of an endothelial cell layer, in this case using multi-plane $\mu$PIV measurements parallel to the cellular surface. Several studies used $\mu$PIV to investigate the fluid slip mechanisms at the wall.\(^{51}\) Tretheway and Meinhart\(^{54}\) used $\mu$PIV to study apparent fluid slip in hydrophobic microchannels and used the results to successively develop a theory for the generating mechanism\(^{53}\) based on the experimental data. The slip length in different glass surfaces was investigated by Joseph and Tabeling\(^{54}\) using a multi-plane $\mu$PIV approach.

To investigate the fluid flow in interfacial or near-wall regions, Zettner and Yoda\(^{55}\) proposed in 2003 to use a PIV technique in combination with evanescent wave illumination from total internal reflection (TIRF), the so-called nano-particle image velocimetry (nPIV). TIRF images are illuminated with the evanescent field of an incident laser pulse impinging upon the glass wall-fluid interface. This allows velocity measurements with an out-of-plane resolution defined by the penetration depth of the evanescent wave, which is typically few hundreds of nanometers. Major sources of inaccuracy for this technique are the non-uniform illumination that presents an exponential decay and non-uniform particle distribution mostly due to near-wall hindered Brownian diffusive motion and electrostatic forces.\(^{22,56–60}\)

nPIV has been recently applied in a wide range of applications. A multilayer nano-particle image velocimetry (MnPIV) was used by Li and Yoda\(^{61}\) to determine velocity gradients within the first 400 nm next to the wall. Ou and Rothstein\(^{62}\) studied the flow kinematics of water past drag-reducing super-hydrophobic surfaces from detailed $\mu$PIV measurements of the velocity profile across the microchannel. Gai \textit{et al.}\(^{63}\) developed a method to simultaneously measure flow velocities in the middle and near-wall of a channel, by selectively switching from the wide field excitation mode to the evanescent wave excitation mode. Single λDNA molecules were then used as a flowing tracer. Near-wall velocity measurements on a fully developed and steady electro-osmotic flow were carried out by Sadr \textit{et al.}\(^{64}\) using nPIV. A method to measure the near-wall temperature was proposed by Guasto and Breuer\(^{21}\) using a TIRF setup and particle tracking. Several authors used nPIV to investigate slip length and velocities in hydrophobic and hydrophilic surfaces in microchannels.\(^{65–67}\)

#### Electrokinetic flow

Electrokinetic phenomena are widely used to operate micro-fluidic devices,\(^{1,68}\) such as electro-osmosis to drive flow in microchannel,\(^{58–70}\) electrophoresis to manipulate particles or molecules.\(^{71}\) When using $\mu$PIV as diagnostic tool in electrokinetic devices the main aspect to bear in mind is that particle migration due to electrophoretic and dielectrophoresis forces is an issue. We can distinguish three different application cases:

(i) Measurement of the absolute motion of particles suspended in the fluid. In this case a traditional $\mu$PIV experiment can be performed with no additional requirements.

(ii) Measurement of the absolute motion of the fluid. In this case the use of $\mu$PIV is limited by the behavior of tracer particles. When high accuracy and resolution are not needed, it is advisable to adopt a dye visualization or molecular tagging velocimetry (MTV) technique.\(^{72}\) When using $\mu$PIV, an earlier calibration of the system is needed. One way is to measure the zeta potential of the particles and channel surface and use it to derive the true fluid motion as demonstrated by Devasenathipathy \textit{et al.}\(^{73}\) Wang \textit{et al.}\(^{48}\) suggested a two color labeled PIV approach to uniquely determine the fluid velocity from the observation of particles without \textit{a priori} knowledge of the electrical properties. Bown and Meinhart\(^{74}\) used a two-color $\mu$PIV approach to measure flow velocities in an AC electrokinetic DNA concentrator. An alternative method to simultaneously measure the zeta potentials of the channel surface and the tracer particles in aqueous solutions using closed and open channel was proposed by Yan \textit{et al.}\(^{75}\)
(iii) The measurement of the relative motion of particles with respect to the fluid is needed. In this case it may be useful to combine μPIV with other techniques such as MTV.

Despite the practical difficulties listed above, μPIV is nowadays widely used as a diagnostic technique in electrokinetic flows, either for the observation and understanding of the underlying physical phenomena or for the characterization of functioning and performances of devices.

An experimental investigation of internal pressure gradients generated in electrokinetic flows with axial conductivity gradients was performed with μPIV by Devasenathipathy et al. Velocity profiles of electroosmotic flow under the influence of Joule heating were measured and compared with numerical results by Tang et al. obtaining a good agreement between experimental and numerical results. Yan et al. presented a model to describe the effect of the finite reservoir size on electroosmotic flow in a rectangular microchannel and used μPIV to verify the validity of the model. The 3D velocity field inside the droplets was reconstructed from 2D μPIV experimental data. Park and Choi developed a method for the simultaneous estimation of inhomogeneous zeta potential and inhomogeneous slip coefficient using velocity measurements by μPIV. Lu et al. recently investigated the flow inside droplets actuated by electrowetting-on-dielectric (EWOD). μPIV was used by Horiuchi et al. to characterize mixed electroosmotic and pressure driven flows in a trapezoidal shaped microchannel.

Studer et al. used μPIV to experimentally characterize the performances of an integrated AC electrokinetic pump in a microfluidic loop. An experimental and numerical verification of the performance of an electrically excited micromixer was presented by Barz et al. using μPIV for the experimental measurement of the flow field within the micromixer. μPIV was used by Huang et al. to characterize in-plane micro-vortices generated by AC electroosmotic flows for stationary or continuous fluid mixing.

We recommend the reviews by Devasenathipathy et al. for further reading.

**Biological flow**

A large part of microfluidic and lab-on-a-chip applications are connected with living organisms. Many examples can be already found in nature such as the micro-flow in blood capillaries or the biochemical reactions and transport phenomena in a single cell.

As a non-tactile optical technique, μPIV is a good candidate for velocity measurements on living organisms with minimal impact on the normal physiological activities. In vivo PIV, the measurement of red blood cell velocity field in arteriole in a rat mesentery, was shown by Sugii et al. using an intravital microscope and high-speed digital video system. Later, in vivo μPIV was used by Jeong et al. to study the movement of liposomes suspended in blood flowing through rat mesenteric capillaries. More recently Poelma et al. presented a methodology for the determination of the wall shear stress in vivo in the vitelline network of a chick embryo using μPIV. Hove et al. measured velocities in a zebra-fish. Vennemann et al. measured the velocity distribution in a beating embryonic heart (Fig. 6). Groenendijk et al. used the results to investigate cardiovascular defects. A review for optical technique for in vivo blood velocity measurement can be found in Vennemann et al.

Besides in vivo experiments, μPIV can find a broad application in lab-on-a-chip and microfluidic devices designed for the in vitro investigation, handling or screening of biological samples. Kim et al. used μPIV to characterize the flow-rate range of a novel microfluidic device for culturing adherent cells over a logarithmic range of flow rates. μPIV was used by Wong et al. to measure the velocity profile and extensional rate of the solvent flow in a microfluidic device for deformation of DNA molecules by hydrodynamic focusing. An in vitro model equipped with a side-view μPIV system was proposed by Leyton-Mange et al. to obtain quantitative flow data over cells adhering to the endothelium. The behavior of physiological saline or in vitro blood flow in PDMS microchannels was investigated by Lima et al. using confocal μPIV. A high-speed μPIV technique was proposed by Sugii et al. to in vitro investigate the rheology of blood flow in microcirculation by measuring both red blood cell velocity and plasma velocity. Several authors used μPIV to study the effect of flow-induced mechanical stimuli on endothelial cells. An example by Rossi et al. is shown in Fig. 7 where...
Mixing

Mixing represents a fundamental process for many technological applications. In most micro-fluidic applications laminar flow is present, which prevents advective mixing such that an interaction only occurs by diffusion. Hence, distances and time scales over which mixing occurs are far beyond practical applicability for micro-fluidic applications. To overcome this ambiguity mixing processes are enhanced. In micro-fluidic devices, this has been achieved in two ways. In passive mixers secondary flow patterns are generated by forcing the laminar fluid streams by channel geometry thereby increasing the contact areas of the mixing agents and reducing the diffusion length. Typical examples are cross-flow mixers, of which the T-joint mixer has extensively been studied, meander or serpentine mixers, or mixers with patterned surfaces such as herringbones, which cause fluid to be locally entrapped in the geometry such that diffusion is enhanced. Active mixers apply localized forcing of the fluid to increase the interface on which diffusive mixing occurs. The variety of approaches is extremely broad, including piezo-electric cantilevers, magnetohydrodynamics, electrokinetic instabilities and many others. Reviews on recent mixing strategies can be found in Stremler et al. and Wu and Nguyen.

μPIV has widely been applied to study either details of flow in mixing devices or the entire fluid field. To investigate the temporal flow field and mixing characteristics in a shear superposition micromixer, Bottausci et al. applied µPIV at the intersection of primary and secondary channels of the mixer at very low Reynolds numbers of Re = 2.6 based on the primary channel geometry. Hoffmann et al. assessed the concentration and velocity field in a passive T-joint mixer at Re = 160 by applying µLIF and µPIV, respectively. While these authors used 2D-2C µPIV, Lindken et al. developed a stereo-microscopic µPIV technique, which allows one to determine the 2D-3C flow field. The technique has been successfully applied to a passive T-joint geometry at Re = 120.

Scanning through the channel geometry allowed the reconstruction of the entire stationary volumetric flow field (Fig. 8).

Two-phase flow

In many technical applications flows of immiscible gas–liquid or liquid–liquid phases are present. While the fluid mechanics of macroscopic dispersed flows have already been studied extensively, there is still need for further research on a micro-fluidic scale. Here, especially the effect of surface wettability and encapsulation, the influence of Capillary number Ca and film formation need further investigation to improve stability and controllability of two-phase flow and the optimization of chemical reactions, diffusion processes or the separation of phases. The miniaturization of analytical instruments yields numerous potential benefits including reduced amount of sample and reagent, high sensitivity, short analysis time, automation of processes, and parallel processing. A very comprehensive review on two-phase flow is given by Günther and Jensen.

The experimental assessment of two-phase flows by µPIV related techniques is a complex task. If the flow inside the droplets is of interest, refractive-index matching needs to be achieved to allow for an undistorted view of the advancing and receding meniscus of the droplet. Particles need to be carefully chosen to guarantee a homogeneous and optimum solvability in either of the two phases and to assure them not to concentrate and agglomerate at the interface.

Kim et al. focused in their paper on the effect of such differences in the refractive indices of two fluids in a two-phase flow on the accuracy of the PIV data and they theoretically assessed the offset in the focal planes of the two fluids and defined condition for the measurement of reliable valid velocity profile across the two-fluid interface.

Günther et al. used micro particle image velocimetry in combination with fluorescence microscopy techniques to investigated segmented gas–liquid flow at low superficial velocities in straight and meandering channel networks to study the residence time distribution for liquid phase reactions. The authors continued their work on the flow field in an integrated micro-fluidic system for mixing of two miscible liquid streams by introducing a gas phase, formation of a segmented gas–liquid flow and final separation of the two phases. Concentration fields and the extent of mixing have been obtained in parallel from pulsed-laser fluorescence microscopy and confocal scanning microscopy measurements.

Kinoshita et al. applied µPIV and confocal PIV to study the internal flow field inside a moving droplet. By phase-averaging and scanning the flow field along the vertical direction, the out-of-plane velocities could be determined analytically based on the continuity equation and the 3D-2C (3-dimensional, 2-component) velocity information. Similarly, Sarrazin et al. measured velocity fields inside the droplet of liquid–liquid flow in rectangular microchannels at different height inside the channel. King et al. found plug flow with no internal circulation. The authors generated aqueous plugs and FC-40 as the segmenting fluid. The authors exhibited a zero gradient velocity profile within the plugs especially at lower velocities and shorter plug lengths. It should be noted that the system contained surfactants. However, surface-active components influence the liquid–liquid interface.
An immobilization of the interface is possible depending on the surfactant concentration. Thus, an explanation for the suppressed internal circulation at low velocities could be an insufficient exertion of shear to an immobilized liquid–liquid interface. In case of a sufficiently large velocity gradient, the interface would be renewed, which leads the onset of the internal circulation. Miessner et al.\textsuperscript{112,113} simultaneously resolved the flow field inside droplets and the immiscible carrier phase in a micro-

\textbf{Fig. 9} (left) Velocity vectors and (right) velocity streamlines of the continuous liquid–liquid droplet flow at various heights in a 100 μm square channel (following Miessner et al.\textsuperscript{113}).
Transition to turbulence in micro-devices

If the Reynolds number becomes large enough, flow in microfluidic devices becomes turbulent. The investigation of transition and turbulence has attracted many researchers in the past years since many fundamental questions are still unsolved. It is e.g. under discussion, whether entrance effects, non-Newtonian effects, wall slip effects or surface roughness effects play a different role in the development of turbulence in microchannels compared to macroscopic flow.

The experimental assessment of turbulent flow in microchannel poses new demands on the correct investigation. First, since turbulent structures scale down with the size of the channel, the integration of the small-scale fluctuations within the PIV interrogation window causes reasonable errors in the determination of fluctuations intensities. For instance, at a typical microchannel height of 100 μm and Reynolds numbers of approximately Re = 2200 (based on the half-height and the bulk velocity), viscous energy dissipation, which occurs roughly at the Kolmogorov scale, appears at approximately 300 nm for the above mentioned flow characteristics. With particle sizes of the same order of turbulent length-scales, low seeding particle density in micro-fluidic applications and a finite spatial resolution of the μPIV measurement is hard to achieve.

Furthermore, wall roughness plays an essential role in the development of the flow field. The critical roughness height, for which the wall can be considered hydraulically smooth, scales with Reynolds number, such that in micro-channels the remaining surface roughness can easily exceed the critical level. This may have been the reason for premature transition to turbulence observed in microfluidic devices by some researchers in the past.

Fully developed turbulent flow requires time and space to develop. That is, entrance lengths similar to those in large-scale flows should be respected.

Unfortunately, many of these requirements have not sufficiently been accounted for, yielding a broad range of partially controversial results reported in the literature on turbulent transition such that there is still need for reliable and careful measurements to further assess the flow characteristics of turbulence and transition at such low geometric dimensions. In the following, some of the results reported in the literature on turbulent transition will be critically discussed.

Liu et al. investigated a planar microscale confined impinging-jets reactor with inlet jet Reynolds numbers ranging from Re = 211 to 1,003. The experimental results obtained by μPIV showed good agreement with numerical data obtained from a k–ε model CFD.

To study the impact of the flow geometry on transition, Hao et al. measured the pressure drop and the velocity field of water flow in a trapezoidal silicon microchannel. μPIV recordings at different positions at Reynolds numbers between 50 and 2,800 were obtained to study the entrance effect in a microchannel At Re > 700, the Δp–Re relationship increasingly deviates from the linear behavior with Re. The authors related this deviation to the entrance effect. From mean flow statistics at the channel centerline the authors reported transition from laminar to turbulent flow to occur at Reynolds number ranging between 1,500 and 1,800, which was consistent with their global pressure drop measurements. In a further study Hao et al. compared water flow in smooth rectangular cross-section microchannels and microchannels with discrete rectangle “roughness” elements, which partly obstructed the channel by approximately 20% such that the transition to turbulence should not be considered due to roughness but due to the 3D flow field. Furthermore, it is questionable, whether or not the changes in the flow field were solely
due to the existence of the surface obstacles or if also entrance length effects played a significant role.

Li et al.\textsuperscript{133} were among the first to study transitional and fully turbulent flow in micro-channels. Turbulence statistics could be obtained from the velocity field data obtained by μPIV. While the authors suggested from their results in this paper that the onset of transition appears at Re = 1,535, they report on slightly higher values for transition in their later publications (Li and Olsen\textsuperscript{144,145}), where they also studied aspect ratio effects and the influence of the hydraulic diameter. These later findings for transition agree well with the results in the literature reviewed in the following. Spatial length-scales of velocity fluctuations were measured by Li and Olsen\textsuperscript{136} along the channel centerlines and at four other locations, and characteristics of turbulent length scales were determined, showing essentially good agreement with the results at large scales.

Transitional flow in microfluidic devices at 1,800 ≤ Re ≤ 3,400 has carefully been investigated by Natrajan and Christensen\textsuperscript{137} and Natrajan et al.\textsuperscript{138} The authors observed a deviation from laminar flow state at Re = 1,900. At transitional flow the flow field shows a strongly intermittent behavior with purely laminar regions and fields of significant departure from laminar behavior. Spatial features of hairpin-like vortices and hairpin vortex packets have been detected.

Wibel and Ehrhard\textsuperscript{139} very carefully performed studies of transitional and fully developed turbulent flow in stainless steel micro-channels for different aspect ratios of 1:1, 1:2, 1:5 at constant hydraulic diameter. During the measurements the mass flow rate, the temperatures at inlet and outlet, the pressure drop, and the time-resolved velocity field (μPIV) have been recorded. These quantities consistently evidence the laminar/turbulent transition in smooth micro-channels to be consistent with macroscopic channels at similar Reynolds numbers.

Sharp and Adrian\textsuperscript{140} also carefully performed pressure drop measurements and μPIV measurements at the centerline of a micro-channel to study the onset of transition in glass micro-tubes. From the μPIV measurements of mean velocity and rms velocity fluctuations they concluded transition not to occur at anomalously low values of Reynolds number.

Complex fluid mechanics problems

To conclude the application chapter, we list a miscellaneous of recent applications in microfluidic and lab-on-a-chip devices in which μPIV was used as diagnostic tool to investigate the fluid mechanics problems involved in the system.

Große et al.\textsuperscript{141} measured the laminar shear flow field in an array of micro-pillars at low Reynolds number to confirm the low and only local intrusiveness of the micro-pillar structures, which the authors apply to measure the spatial two-directional wall-shear stress distribution in turbulent flows. The effect of thermophoresis in a micro-channel particle separator has been investigated by Geelhoed et al.\textsuperscript{142} μPIV measurements were performed to measure the particle migration induced by the temperature gradient field. To investigate the performance of a piezoelectric (PZT)-micropump, Sheen et al.\textsuperscript{143} studied flow characteristics at the outlet of the micro-fluidic device and around trapezoid obstacles placed in the channels leading into and out of the pump chamber. Since pumping frequencies in the order of several kHz were applied, the μPIV recordings were synchronized and phase-locked with the pumping mechanism. Laminar flow in a rectangular microchannel with microheater devices was investigated by Park et al.\textsuperscript{144} to study the effect of the additional heat flux on the laminar flow characteristics. Pressure drop and flow rate measurements were accompanied by μPIV investigations of the velocity fields along the microchannel at various wall temperatures. Whether or not the authors compensated their PIV system for changes in the refractive index of the working fluid with temperature was not mentioned in the paper. Hagsäter et al.\textsuperscript{145} used μPIV analysis in combination with images of transient particle motion for experimental studies of acoustic radiation forces and acoustic streaming in microfluidic chambers under piezo-actuation in the MHz range. The experiments were supported by numerical solutions of the corresponding acoustic wave equation (Fig. 10). The flow in a microfluidic sheathing structure has been measured by Klank et al.\textsuperscript{146} by application of a 2D and 3D-translation μPIV approach. From the 3D-translation μPIV results the authors were able to reconstruct a 3D-3C volumetric steady flow field, although due to conceptual restriction of the approach\textsuperscript{147} there is a unacceptable error in the reconstruction. Meinhart and Zhang\textsuperscript{148} applied phased averaged μPIV to measure instantaneous velocity fields in a commercial inkjet printhead during all four primary phases of the ejection cycle. Instantaneous velocity fields of the flow inside the cavity and of the mesiniscus shape. The authors reported flow with velocities reaching up to 8 m/s, Reynolds numbers of Re = 500 and accelerations of up to 70,000 g. Hohreiter et al.\textsuperscript{149} used μPIV to measure temperatures in a fluid volume with an accuracy of 3 K. The technique is based on the premise that Brownian motion will cause width-wise broadening of the cross-correlation peak. A correlation-based PIV algorithm detects the magnitude of Brownian particle motion and can be used to determine the temperature of the fluid. Park et al.\textsuperscript{150} used a similar approach based on Particle Tracking.

![Fig. 10](image)

**Fig. 10** Experimental study of acoustic radiation forces and acoustic streaming in microfluidic chambers under piezo-actuation in the MHz range. The yellow μPIV-vectors indicate the initial bead velocities pointing away from pressure anti-nodes and the picture underneath the PIV-vector plot shows the particles (black) gathered at the pressure nodal. On the bottom right corner is shown the numerical simulations in the 2D chamber model of the corresponding acoustic pressure eigen-modes. (From Hagsäter et al.\textsuperscript{145} – Reproduced by permission of The Royal Society of Chemistry).
Summary and conclusions

In this review we focus on micro-scale Particle Image Velocimetry (μPIV) in theory, practical issues and applications. Over the past years μPIV has become the most popular velocimetry method at sub-millimeter scale. Compared to other methods presented in the introduction experience on various applications exists and complete systems are commercially available. Albeit, one should keep in mind that like with all image-processing based methods specialized experience of the user is needed for a reliable measurement.

In chapter 2 the μPIV technique was described. The measurement principle of μPIV is well-developed and well-understood. Specialized PIV-cameras and light sources became commercially available in the last years. A theoretical description of the evaluation technique is available. The description of the depth of the measurement volume is still a controversially discussed issue of research. Several contributions were added in the last years but a universal formulation is still missing. The μPIV user should be aware that the calculation of the depth of correlation is only an estimate.

In the μPIV chapter we have described a set-up, which is optimal to our opinion. Several variations are possible. In particular one may be tempted to use larger or faster cameras, but the essential characteristic of the imaging device is photon sensitivity and signal quality. High-speed cameras have been applied in microflows, but the measurements suffered from low image quality. The limiting factor for the measurement of high-speed flows at high magnifications is not the frame rate of a camera, but the interframe time (the time needed for the readout of the image). High-speed cameras have typically long interframe times and are not suited for fast flows. Despite their low photon sensitivity they are the best choice for unsteady flows. Alternative illumination sources like IR-lasers or LEDs reduce the costs of a system, but the signal quality is reduced. In general one should use fluorescence imaging if possible in the experiment, because by this way scattered light from channel walls, which interferes with the particle signal, is removed and does not contribute to the image noise. Other variations make the μPIV set-up more complex. High-speed Confocal Laser Scanning microscopy (CLSM) has been used in order to reduce the depth of field of the microscope with the aid of a pinhole in the detection beam path that severely reduces the amount of light reaching the camera. As a consequence larger particles are needed that increase the depth of correlation again. Furthermore, the high-speed CLSM systems allow no short time interval between two consecutive recordings, which reduces the applicability of the method to low velocities in the order of nanometer to micrometer per second.

The chapter on practical guidelines may serve as a guide for the beginner and may offer suggestions to the experts. For example, the insertion of a diffuser plate in the illumination beam path is a cost-efficient method to reduce speckle noise from μPIV-recordings and to protect the objectives from laser damage. One of the open issues in practical applications is the behavior of tracer particles in electrokinetic flows. Charged particles experience a force in an electric field. One solution may be to generate neutrally-buoyant tracer particles with no zeta potential in a buffer solution with high pH (needed for efficient electroosmotic transport).

In the application chapter we give an overview of μPIV experiments in various applications. For the investigation of two-phase flows μPIV has the advantage that both phases can be observed simultaneously and the measurement position relative to the two-phase interface can be determined from the recordings. The large number of publications in this field shows the importance of the measurement technique for micro-scale multiphase flow research. Precise velocity measurements close to surfaces and in electrokinetic flows are difficult to accomplish due to questionable particle flow fidelity. Especially wall slip measurements with μPIV are controversially discussed. It appears that the more careful wall slip is measured, the smaller the measured wall slip is. There is a need for further verification measurements. A few years ago publications suggested an onset of turbulence in microchannels at lower Reynolds number. Recent measurements with high accuracy do not support this suggestion. The use of μPIV in biomedical applications is growing fast and has generated new insight in biological and medical problems. Mixing at microscale is an important issue and μPIV is often applied for the characterization of micro-mixers.

Although μPIV is well-established and several companies offer reliable commercial μPIV systems, there is active research towards novel methods and applications. Future developments of the μPIV method may be in direction of easy to apply three-dimensional velocimetry methods, either by defocusing stereoscopic or holographic imaging. A recent review on advanced particle-based velocimetry techniques is given by Lee and Kim. A reliable method for μPIV in gas flows in microchannels is missing. Today the working fluid is a liquid. Solutions for gas flow applications are expected. New applications can be expected in life science research, too. The application of the method to non-transparent material may be further developed e.g. with infrared light sources or Magnetic Resonance Imaging (MRI). Since the μPIV method is universal in the application and accurate and reliable to use we expect various new developments and applications.

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