Depth resolution by means of helical wavefronts for particle tracking applications

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We present a technique for 3D measurements of flow fields. A double-helix point spread function is generated by a spiral phase mask with a spatial light modulator (SLM). Double images of seeding particles are evaluated within the depth of field (DOF). The orientation of the axis between those double images changes in dependence on particle location within the DOF allowing robust axial localization.

1 Introduction

Particle image velocimetry of particles and biological cells is a common method for micro- and nano-fluidic analysis [1]. For moderate particle/cell densities particle tracking velocimetry (PTV) can be applied [2]. Depth information within the DOF of a microscope objective is usually gained by scanning along the optical axis, e.g. by a piezo-electric element or an adaptive lens [3]. Possible solutions for this drawback are common non-scanning methods as the astigmatic [2], the biplane approach [5] or the DH-PSF method [4, 6].

2 PTV in a micro-channel

Numerous established techniques exist for fluid flow measurements in micro-channels [1]. We intend to perform 3D particle tracking velocimetry (3D-PTV) by generating a double-helix point spread function (DH-PSF). The DH-PSF results in a double-image for each seeding particle. The orientation of the double-image gives information on its position along the optical axis. A reasonable advantage of this technique is gathering depth information without scanning along the optical axis (z-stacking). In a common way a parabolic flow profile was measured within a 400 µm thick micro-channel as it is shown in Fig. 1. The measurement was done by performing one PTV evaluation for each z-value within the thickness of the micro channel. This kind of procedure is time-consuming, yields large amount of partially redundant video data and needs evaluation for each z position. Our intent is to measure only one set of video data in order to extract full flow-field information $\vec{v}(\vec{r},t)$ with $\vec{r}(x,y,z)$ in one shot, i.e. all three velocity components within a volume that is defined by the field of view FOV and the DOF of the microscope objective: FOV*DOF. For tracking two equidistant spots representing one particle, a Gaussian mask correlation algorithm was applied. The rotational orientation of the double-images is referred to a calibrated reference orientation (convenient here is the central plane of the micro-channel thickness, see also Fig. 1).

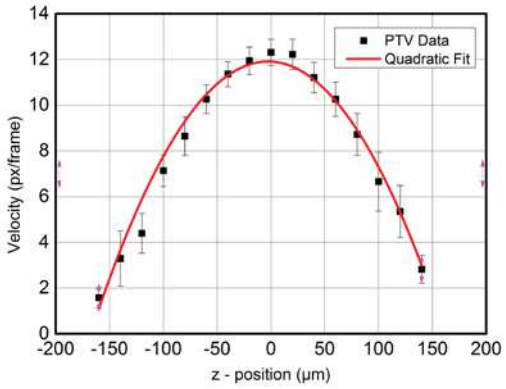

***Fig. 1** Parabolic velocity profile of the flow field in a micro-channel measured by z-stacking of individual PTV data. The orange rectangle (+- 60 µm) indicates the accessible DOF for instantaneous 3D information that can be derived from the data shown in Fig. 4c.*

3 Setup

The measurement setup consists of mainly five components as it is indicated in Fig. 2. The image of the seeding particles from a micro-channel is transferred via an SLM performing phase-only modulation with a spiral phase mask. The modulated image is recorded by a CCD camera. The spiral phase mask can be optimized for certain particle sizes and magnifications in order to gain optimal contrast for the double-images. The change in orientation angle $\mathrm{d}\psi$ with distance change dz along the optical axis can be expressed in the following way [6]:

$$\frac{\mathrm{d}\psi}{\mathrm{dz}} = \frac{\pi(\mathrm{NA})^2}{\lambda \mathrm{N} \Delta \mathrm{l}} \qquad (1)$$

Important parameter are the numerical aperture NA of the optical system, the utilized wavelength λ, the number of radial zones N and the topological charge between neighboring zones Δl on the phase mask (see also inset of Fig. 2). For Δl=2 a double-image

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is generated. For increasing N the separation of the spots increases while the overall contrast is reduced.

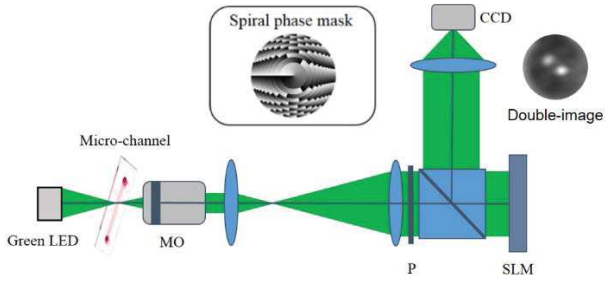

Fig. 2 *Experimental setup for micro-channel flow measurements. A 400 µm thick micro-channel is illuminated with a LED from the backside. The image of the microscope objective is transferred via a SLM loaded with the indicated spiral phase mask. Double images of each seeding particle appear on the CCD camera.*

4 Results

In Fig. 3 static particles are shown that are attached to the micro-channel inner plastic side in an air environment (10 µm sized, 40x). By switching on the SLM each particle is converted into a double-image of its own structure. Depending on the magnification one can observe individual structures doubled. In Fig. 3a the inner center of the particle sphere is appearing twice for both particles. Whereas in Fig. 4 the boundary of the particle is reproduced in a double-image. By identifying the orientation of the double-image one is able to deduce the location of a particles along the optical axis z. A calibration measurement is shown in Fig. 3. Negative orientation angles show larger deviation than positive ones due to aberrations that can be compensated in principle by applying adaptive optical techniques [6].

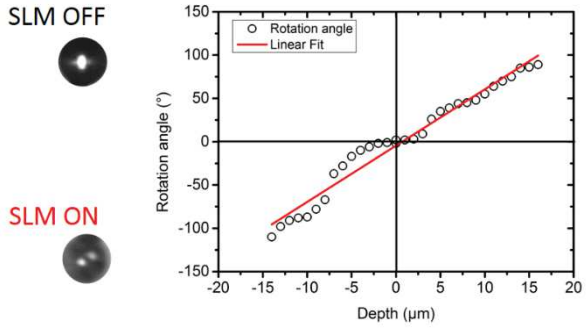

Fig. 3 *Seeding particle of 10 µm size with increased transmission in the center. Double images are generated for the center of the particle. The calibration curve was determined by driving the micro-channel on a translation stage through the focus.*

In Fig. 4 are shown 2 µm sized particles with only 10x magnification. The particles levitate in water while exhibiting Brownian motion in x,y and z direction (indicated by weak rotation of the orientation in a random manner). After contrast enhancement (Fig. 4a) and inverting grey-values, a common PTV Gaussian mask algorithm can detect the double-images. Five particles were detected in different locations along the optical axis as it is demonstrated in Fig. 4b. In the same experimental setting it was possible to record a video of a syringe pump driven flow. Fig. 4c only shows part of the recorded data in a narrow window in order to demonstrate that two particles (green) can be identified in a certain depth passing by two other particles (red) located at another depth of the micro-channel. These data demonstrate that the flow-velocity in different depths can be measured in one record by applying the DH-PSF method. The measured range of depth was estimated to be +- 60 µm (see also Fig. 1). In summary, we showed 3D particle localization of static and flowing particles in a micro-channel using high NA microscope objectives without scanning.

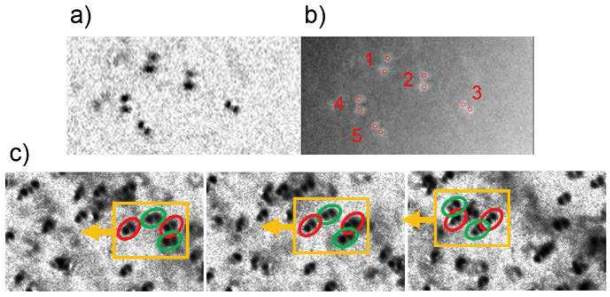

Fig. 4 *a) Double-images of five particles performing Brownian motion. b) Identification via Gaussian mask correlation algorithm. c) Micro-channel flow. Green indicated double images pass by red indicated ones while levitating in a faster layer of the flow (compare orientations).*

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