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Dynamic phase-contrast stereoscopy for microflow velocimetry

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Abstract A nonlinear dynamic phase-contrast stereoscope has been developed for the measurement of all three velocity components of a microfluidic flow field. The stereoscope system captures simultaneously two images of different off-axis views of the same region of interest in a microflow, seeded with tracer particles. Two independent photorefractive two-beam coupling novelty filters, one in each stereoscope channel, are employed to enhance the contrast of tracer particle images. A subsequently applied particle tracking algorithm extracts the velocity information from the images, and in first experiments the axial velocity components could be determined with an error of less than 5%. Finally we report on the determination of the velocity field in a rectangular microchannel with a 170 μm high microstep with the dynamic phase-contrast stereoscope.

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1 Introduction

Microfluidic systems, such as micro total analysis systems (μTAS) [1] or lab-on-a-chip devices (LOC) [2], have a great potential for applications in micro scale chemical synthesis and medical diagnostics [3]. The knowledge of occurring microflow velocities in these devices is an important parameter for the characterization and control of their functionality.

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Micro-particle image velocimetry (μPIV) is a well established technique for the determination of two-dimensional velocity information of microflows [4–6]. In recent years, several strategies to measure all three velocity components in microfluidic systems were reported. Based on their macroscopic counterparts, holographic [7, 8], defocussing [9–11], and stereoscopic techniques [12–14] were applied to the microscopic scale. Because the working principle of all these techniques is based on the detection of tracer particles, a good particle contrast is mandatory. Therefore, most studies were realized by using particles labeled with fluorescent dyes. Theses fluorescent dyes can cause problems due to aggregation [6] or biotoxicity [15]. Furthermore, interesting microfluidic systems, such as microorganismic-induced flow fields [16] or blood vessel flows [17] would be hampered by introducing an artificial, fluorescent seeding.

To utilize the capability of stereoscopic μPIV for analyzing the full velocity field in microfluidic systems without the use of fluorescent tracer particles, we combined it with a nonlinear dynamic phase-contrast technique. It transfers temporally changing phase or amplitude information of an object into intensity information, thus allowing for real-time phase visualization [18] and measurement [19–21]. In comparison to conventional phase-contrast techniques the dynamic phase contrast is not a Fourier filter in the spatial domain, but a dynamic filter in the time domain with adjustable filter characteristics. Thus the method is often described as a temporal high pass filter, which detects temporally dynamic signals while suppressing the static background (novelty filter) [18, 22, 23]. The particular features of this technique leads to several applications of the method, ranging from bio-compatible dynamic phase-contrast microscopy [16] and holographic phase-contrast optical tweezers [24] to micro-flow velocity field analysis [25, 26] and micro-mixing visualization [27, 28].

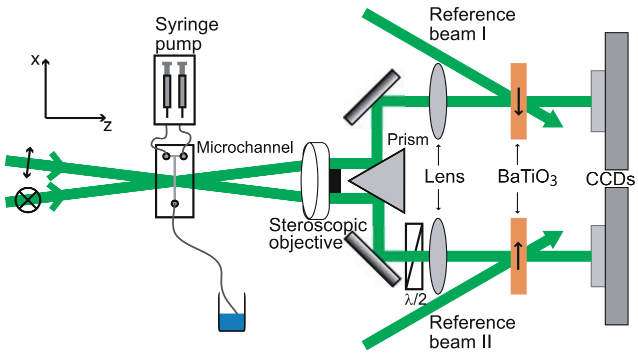

Fig. 1 Sketch of the dynamic phase-contrast stereoscope. Two orthogonal polarized beams illuminate a microfluidic device. The $BaTiO_3$ crystals, in novelty filter configuration, provide the dynamic phase-contrast images, which are captured by two CCDs

In this article, we present the development of a stereoscopic dynamic phase-contrast μPIV system. After the description of the experimental implementation we will discuss the three-dimensional intensity response of the system for transparent tracer particles. Finally, we will show a measurement of all three velocity components in a rectangular microchannel with a microstep.

2 Experimental implementation of a dynamic phase-contrast stereoscope

The dynamic phase-contrast stereoscope is realized as depicted in Fig. 1. The microfluidic device is illuminated by two orthogonal polarized laser beams of an frequency doubled Nd:YAG Laser ($\lambda = 532$ nm). The light coming from the flow field is collected by a stereoscope objective ($f =$ 75 mm) and the two beams are reflected in opposite directions by a reflecting prism. These signal bearing beams are then imaged by additional lenses onto two cameras. In front of the cameras the signal beams interfere inside two barium titanate crystals ($BaTiO_3$) with coherent reference beams. These two $BaTiO_3$ crystals act as two independent optical novelty filters and realize the dynamic phase-contrast functionality in this setup. In steady state the output equals zero intensity, due to the energy transfer from the signal beam to the reference beam. Any novelty within the input signal is instantaneously detected as an intensity peak in the output signal. Moreover, the signal is optically amplified, resulting in an optimized contrast of tracer particles. The novelty may be a change of amplitude or phase of any part of the input image [20]. After detecting an input change, the output falls towards zero intensity within a characteristic time τ of the photorefractive material [29], which can be adjusted by the reference beams intensities. For typical microfluidic velocities of several hundred micrometers per second, τ should be chosen in the range of seconds in order to avoid for trail or contour formation [20].

The high-contrast particle field images are captured by two CCD cameras simultaneously, and a particle tracking algorithm, adapted from Guezennec et al. [30], is applied to extract all three components of the particle velocities (particle tracking velocimetry, PTV). The calibration of the stereoscopic system is done by a calibration target, which is similar to the microfluidic devices used in the measurements [12]. Calibration images are taken at varying displacements from the focal plane, and these images are then used to create a linear transform for conversion between the image and object spaces.

3 Particle imaging

The advantage of the nonlinear dynamic phase-contrast to optimize the particle visibility for PIV or PTV measurements can be seen in Fig. 2. Here a typical flow seeded with 10-μm-thick polystyrene particles obtained by one channel of the stereoscopic system is depicted. On the right an intensity cut along the yellow line shows the intensity profile of one typical tracer particle. The dynamic phase contrast eliminates any detrimental background noise and highlights the moving particles.

For the investigation of three-dimensional flow fields it is important to know the response of the system for particles at different axial positions. Therefore, we fixed polystyrene particles ($d = 4$ μm) on a microscope slide and covered them with a cover slip. Then we observed the intensity response of the system when moving the particles in axial direction (z-axis). In measurements with blocked reference beams, meaning without dynamic phase contrast, we just moved the microscope slide in axial direction by a stepping motor. As the dynamic phase contrast only detects moving objects, we moved the particles in experiments, with both reference beams on, simultaneously in the axial and in transverse direction by two stepping motors.

In Fig. 3 the top row shows the intensity distribution in the xz-plane without dynamic phase contrast for the two stereoscope channels. In contrast to fluorescent particles, these transparent micron spheres show an asymmetric intensity response due to focusing effects and Mie scattering [9]. For conventional PIV and PTV algorithms this intensity distribution would make reliable velocity measurements impossible. The lower row in Fig. 3 shows the intensity response of the dynamic phase contrast for the same particle as in the upper row. It can be clearly seen, that the intensity distribution is, due to the excellent phase sensitivity, symmetric and thus more suitable for PIV and PTV evaluations. The particle signals can be detected over a depth of more than 300 μm, thus allowing a large measurement volume. The noisy background in theses measurements is due to the controlled movement of fixed particles by stepping motors

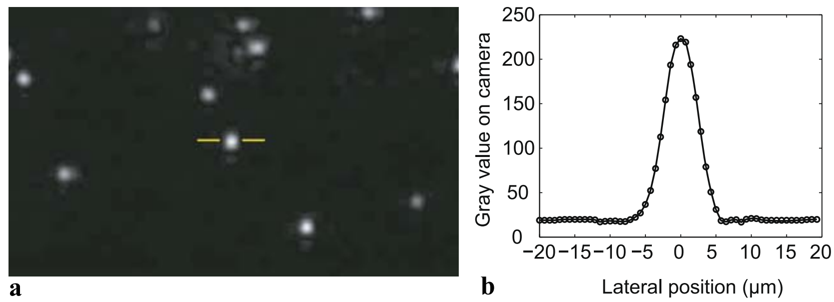

Fig. 2 (**a**) Typical image of tracer particles ($d = 10$ μm) in a microflow. (**b**) Intensity profile of one particle (along the *yellow line* in (**a**))

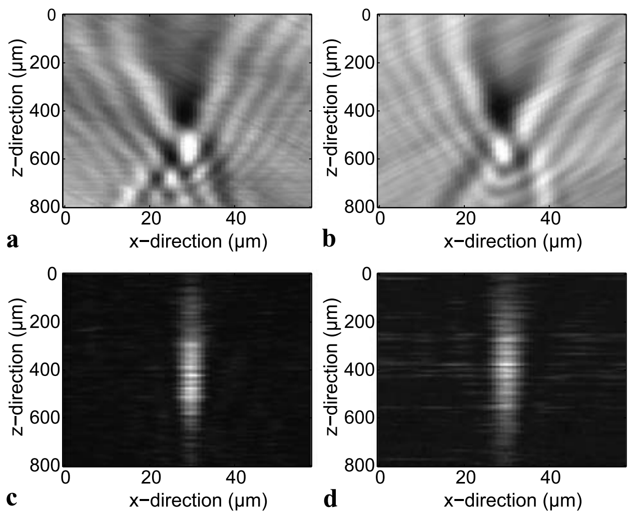

Fig. 3 Axial intensity response of the system without ((**a**) left stereoscope channel (**b**) right channel), and with dynamic phase contrast ((**c**) left channel, (**d**) right channel). The particle diameter is 4 μm

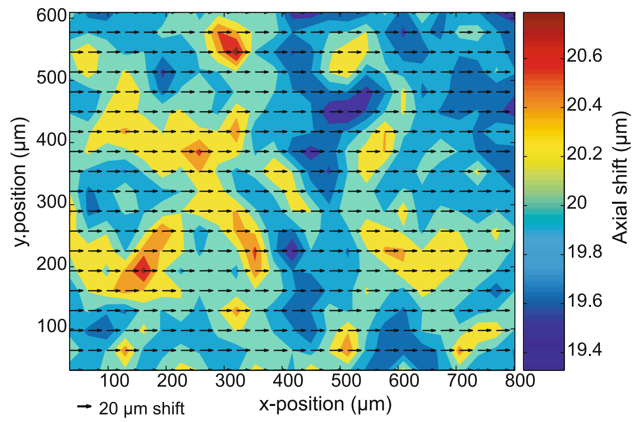

Fig. 4 Velocity profile of tracer particles ($d = 4$ μm) fixed on a microscope slide. The given velocity was 20 μm/sec in axial and lateral direction. The *arrows* show the lateral and the background color encodes the axial velocity

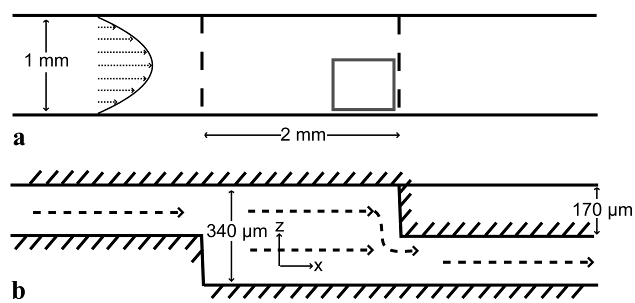

Fig. 5 (**a**) Top view of the channel. The *little box* marks the measured area depicted in Fig. 6. (**b**) Side view of the channel. The sketch of the channel is not true to scale, and important lengths are specified

and does not occur for particles in flow fields (see Fig. 2). In summary, the features of dynamic phase contrast stereoscopy for velocity field analysis are an optimized contrast of tracer particles and a symmetric axial intensity response over a long axial distance.

4 Velocity field determination of three-dimensional flow fields

In order to test the validity of the calibration scheme and the PTV algorithm, we first measured the controlled movement of particles fixed to a microscope slide. We induced a movement of 20 μm/sec in axial and in one lateral direction by the use of micron-stepping motors. The result of the measurement with the dynamic phase contrast stereoscope is depicted in Fig. 4.

The maximum deviation of the measurement from the predetermined velocity is less than 3% in lateral and less than 5% in axial direction. For comparison, Bown et al. report a RMS error of 3% in lateral and 7% in axial direction for their system [12].

For the application of the dynamic phase contrast stereoscope to real three-dimensional microflows we constructed a microchannel with two incorporated steps (Fig. 5). The step height is 170 μm, and the full channel height is 340 μm at a channel width of 1 mm. The fluid was seeded with 10-micrometer-thick particles and driven by syringe pump in order to control the flow velocity.

In Fig. 6 the velocity in the central axial plane in front of the second step is depicted with a resolution of 100 μm × 100 μm × 60 μm (see small black box in Fig. 5a). In the lateral velocity profile, illustrated by the arrows, the decrease

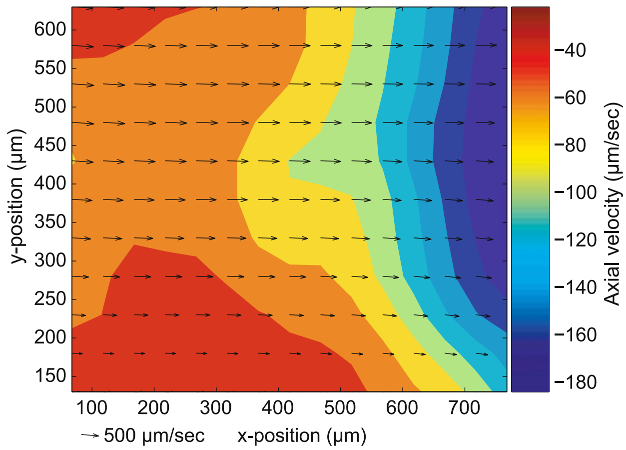

Fig. 6 Velocity profile of the central plane in the marked region of Fig. 5. Lateral velocities are illustrated by the arrows and the axial velocities are color encoded

of the flow velocity near the channel wall is visible. The maximum lateral velocity in the middle is about 510 μm/sec. The axial velocity is encoded as background color. Again the maximum velocities occur in the middle of the step with a value of about 180 μm/sec. The rather low resolution is due to the averaging of velocities for several particles in a 100 μm × 100 μm × 60 μm volume in order to get a dense velocity field. This averaging is common to all PTV evaluations, but could be optimized by using more powerful tracking algorithms [12]. Because the aim of this article is not the optimization of PTV algorithms, but the demonstration of the usability of dynamic phase contrast for stereo μPTV measurements, we did not implement a high performance PTV algorithm at this state. Nevertheless, the system is capable to measure velocities up to 1 mm/sec with an axial resolution of 60 μm by detecting non-fluorescent, transparent tracer particles with an optimized contrast and axial symmetric intensity response.

5 Conclusion

A dynamic phase contrast stereoscope was developed to measure all three velocity components in microfluidic flows. Dynamic phase contrast has three interesting characteristics for microflow analysis: Firstly, it can visualize non-fluorescent, transparent tracer particles with high image contrast. Secondly, it shows a symmetric intensity response for axial movements of tracer particles, and thirdly the signal of tracer particles can be tracked over 300 μm in axial direction, giving a large observation volume. First measurements of microfluidic flow fields were performed. The error of determined velocities is less than 3% in lateral and less than 5% in axial direction. Finally, the dynamic phase contrast stereoscope was used to measure the velocity field of a microchannel with a resolution of 100 μm × 100 μm × 60 μm.

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