Nonlinear Dynamic Phase Contrast Microscopy for Microflow Analysis

Frank Holtmann, Mike Woerdemann, and Cornelia Denz

Abstract. For the investigation and control of microfluidic systems innovative microscopy techniques are needed which can comply the requirements regarding to sensitivity and spatial as well as temporal resolution. A promising approach for this challenge is nonlinear dynamic phase contrast microscopy. It is an alternative full field approach that allows to detect motion as well as phase changes of unstained micro-objects in real-time without contact and non destructive, i.e. fully biocompatible. In this contribution we will present the dynamic phase contrast technique and its applications in micro flow velocimetry and micro-mixing analysis.

1 Introduction

Micro- and nanofluidic devices are of growing interest in the fields of micro scale chemical synthesis and medical diagnostics [25]. The ability to control small amounts of fluids (10^{-9} to 10^{-18} litres) has induced several promising strategies such as micro total analysis systems (μTAS) [15] or lab-on-a-chip devices (LOC) [7]. For an optimization of the chip design its flow characteristics have to be analyzed. Important parameters for this characterization are velocities occurring in the flow field and the mixing quality in reaction chambers.

Techniques for determining the velocity of fluid flows often rely on seeding the fluid with tracer particles [1]. Common velocimetry techniques are particle image velocimetry (PIV) and particle tracking velocimetry (PTV). In microfluidics the intensity of reflected light from tracer particles is usually too low for PIV evaluations [23], so that mainly fluorescent particles are employed [21].

The second important issue in microfluidics is mixing of reagents [23]. The desired control of quality and temporal behaviour of mixing requires a non-intrusive,

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label-free analysis tool with high spatial and temporal resolution. Although fluorescence based techniques such as laser induced fluorescence have a great potential according to chemical sensitivity and spatial resolution [6] they can cause problems due to biotoxic implications [18, 23].

Here we present a different approach that can overcome the above mentioned challenges. A dynamic phase contrast (DynPC) microscope transfers temporally changing phase or amplitude information of an object into intensity information. This microscopy technique is a nonlinear optical filter, applied to the microscope image before its detection on the camera, thus allowing for real-time phase visualization [2] and measurement [11]. Hence the phase changes introduced by fluid flows or moving tracer particles can be visualized without using any fluorescent markers [13]. After an introduction of the working principle and the basic characteristics of nonlinear DynPC microscopy we will present applications in microfluidic velocimetry and quantification of micro-mixing processes.

2 Nonlinear Dynamic Phase Contrast Microscope

Nonlinear dynamic phase contrast microscopy is based on real-time holographic interferometry [8] by photorefractive two-beam coupling [28]. The image information transmitted by an optical system is used as the signal beam which is overlaid with a coherent reference beam within a photorefractive material. It is realized in a modular design, by mounting the photorefractive filter between a microscopes output and a camera (figure 1) [3]. In steady state the output equals zero intensity, due to an energy transfer from the signal to the reference beam. As a direct result of the interferometric nature of two-beam coupling, any novelty within the input signal instantaneously is detected as an intensity peak in the output signal. The novelty may be a change of amplitude or phase of any part of the input image [11]. After detecting an input change, the output falls towards zero intensity within a characteristic time τ which is determined by the grating time constant τ_g of the photorefractive material [29]. Although the decay of output intensity is known to be best described by an exponentially decaying term weighted by an infinite sum of Bessel functions [10], in most experimentally relevant situations it can be estimated very well by a purely exponential decay with an effective time constant τ [22].

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) [2, 4]. The particular features of this technique leads to several applications of the method, ranging from bio-compatible dynamic phase contrast microscopy [5, 20] and holographic phase contrast optical tweezers [27] to micro-flow velocity field analysis [26] and micro-mixing visualization [9, 13]. Detailed investigations of micro-organismic induced flow fields can be found in references [19, 30]. Here we will concentrate on the analysis of

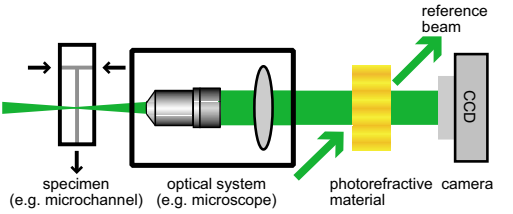

Fig. 1 The modularized DynPC microscope consists of an optical system (e.g. microscope), which images the object under investigation onto a camera and the photorefractive filter, where signal and reference beam interfere, which is mounted between the optical system and the camera

microfluidic velocity fields and the measurement of concentrations in micro-mixing devices.

3 Features of Nonlinear Dynamic Phase Contrast Microscopy

In the last section we described nonlinear DynPC microscopy as a temporal filtering system, sensitive for amplitude and phase changes. Therefore the temporal response of the system is of utmost importance. Figure 2 shows the intensity response for rectangular objects, moving with velocity v and length l from left to right through the field of view. The object velocity decreases from a) to c). If the ratio v/l is very large in relation to the inverse time constant of the system $1/\tau$ (figure 2 a)), the object shape is reproduced exactly, as in conventional microscopy, with a spatial resolution down to the diffraction limit. However, the contrast is enhanced significantly due to a suppression of any static background and phase information is transferred quantitatively in intensity information. Both features will be described in detail in the following sections. If, on the other hand, τ is chosen such that $v/l \approx 1/\tau$, *trail formation* is exhibited (figure 2 b)). Intensity and length of the trail depend on the velocity of the object [11, 13] and consequently can be utilized for measurement of the object velocity [26]. For ratios of v/l much smaller than $1/\tau$, contour formation is visible (figure 2 c)) . This feature can be used for optical edge enhancement for edges perpendicular to the direction of motion [22]. The three cases can be realized experimentally by changing the photorefractive time constant τ. This is possible from milliseconds to hours by adjusting the reference intensity [11, 13].

Fig. 2 Intensity response for rectangles, moving from left to right. Three cases are shown for different velocities v in relation to the system time constant τ for objects of length l. a) $v/l \gg 1/\tau$, b) $v/l \approx 1/\tau$, c) $v/l \ll 1/\tau$

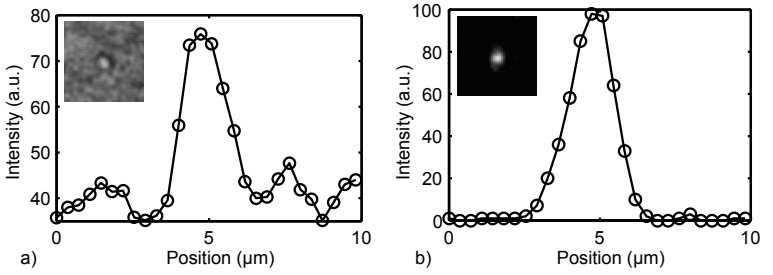

Fig. 3 Particle contrast for a polystyrene sphere with a diameter of 2 μm (images shown in the inset). Intensity profile under bright field illumination (left), and as observed with dynamic phase contrast (right). Lines are guides to the eye

3.1 Contrast Enhancement

The contrast enhancement feature of the dynamic phase contrast microscope can be demonstrated with a defined particle as used for PIV measurements. Figure 3 shows the intensity profile of a polystyrene particle (d = 2 μm) that is driven through the field of view by a laminar flow. In comparison with the conventional bright field image, a significant improvement (here by a factor of 3) of particle contrast is observed due to the phase sensitivity of the dynamic phase contrast microscope and the suppression of any background with slower dynamics than the particle velocity. The factor of contrast enhancement is depending on the phase shift by the particle and the ratio of signal and reference beam intensity. Optimization of contrast is achieved already at very low light intensity in the order of micro or even nano watts, due to the optical amplification by the reference beam which is not transmitted through the sample and thus does not contribute to the total light pollution.

3.2 Spatial Resolution

The spatial resolution of a microscopic technique is usually derived from the *point spread function* of the system. Therefore, a point source is imaged and the width of its airy disk is taken as a measure for the spatial resolution. For larger objects, e.g. μm thick tracer particles or biological cells, Mie scattering is getting relevant and has to be taken into account. For microfluidic applications this was investigated by Ovryn in detail [17]. Therefore here we concentrate on the system response for particles in the μm-range. The nonlinear dynamic phase contrast microscope detects moving objects or changes in phase and amplitude of an object. Static scenes are completely suppressed. Thus the definition of resolution is only well defined for moving objects. The lateral resolution of dynamic phase contrast microscopy, as can be seen in figures 2 and 3, is the same as in conventional microscopy. In contrast to conventional phase contrast microscopy we introduce no aperture in the Fourier plane and therefore we can utilize the full numerical aperture of the microscope

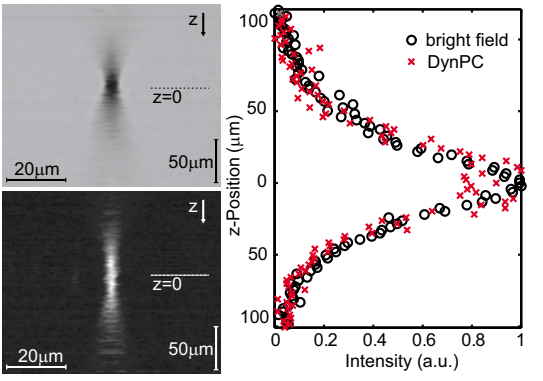

Fig. 4 Z-stack of a 5.6 μm thick, absorbing particle observed by conventional (top) and dynamic phase contrast microscopy (bottom). On the right the axial intensity profile is plotted. Intensities are normalized to the maximum

objective. For the axial resolution one can distinguish two different cases. The first one occurs when using completely absorbing tracer particles. A typical z-stack for a 5.6 μm thick completely absorbing particle is displayed in figure 4. For this measurement the particle is moved laterally and than an image is taken for every axial plane. Stacking the lateral intensity profile of the particle in one image yields the z-stack. Both systems show a symmetric response when focussing through the particle. For comparison of the z-stack intensity profile we normalized the intensity to its maximum. The intensity profile of the z-stack shows, that the axial width of the particle image is the same for both systems,which implies the same axial resolution.

The more interesting case is given when using transparent particles. Then Mie scattering and focussing effects by the particle come into account. In figure 5 it can be seen, that the maximum intensity is not congruent with the particle position. In μPIV or μPTV applications this effect can introduce an offset of several microns in the estimated axial position of the particle [17]. In DynPC microscopy this effect is suppressed and the phase shift of the particle is more important for the axial response of the system, yielding a symmetric response. This behaviour can be utilized for determining the correct axial position in scanning PIV or PTV measurements.

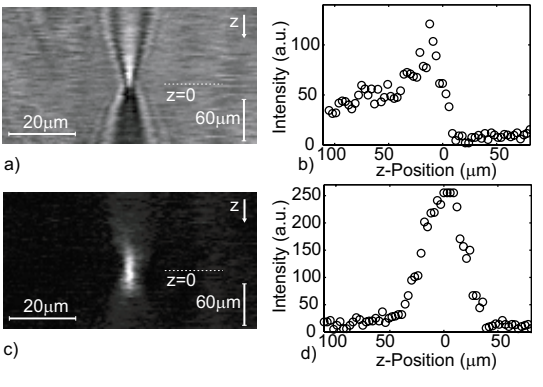

Fig. 5 Z-stack of a 4.4 μm thick, transparent particle observed by conventional coherent (a)) and dynamic phase contrast microscopy (b)). On the right the axial intensity profile is plotted

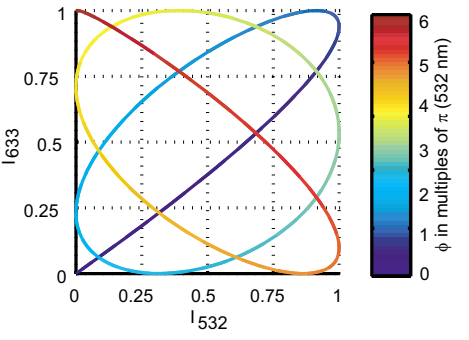

Fig. 6 Two-wavelength phase transferfunction of DynPC microscopy. The intensity tuple I_{532} and I_{633} is a direct measure of an introduced phase shift ϕ

3.3 Phase Sensitivity

The output intensity I_{out} of nonlinear DynPC microscopy is directly dependent on phase variations introduced to the signal beam. Based on theoretical and experimental investigations, the dependence of the dynamic phase contrast output on a phase shift ϕ can be described by $I_{out} = I_{max} \sin^2(\phi/2 + \chi - \pi/2)$ where I_{max} is the maximum signal intensity used in the system, which is fixed during the measurement process [13]. The extra phase term χ takes a possible grating phase shift into account, either introduced by the photorefractive material or an external phase shift applied to one of the two beams. For a measurement of phase shifts, the phase transfer function (PTF) of the system has to be determined experimentally by introducing known phase shifts onto either the signal or the reference beam. With this PTF a simple look-up table can be created to determine an unknown phase shift. The simplicity of this dependence allows real-time measurements of phase changes such as those introduced by density changes in fluids [13]. The output intensity I_{out} shows that the PTF has a quadratic sinusoidal dependence on the introduced phase shift ϕ. This causes a limited range of uniqueness within the interval from zero to π radians. For measurements of larger phase shifts in realistic liquid and gaseous fluid flows, this limitation has to be eliminated.

A phase triggering method can be employed to extend the phase measurement range to 2π radians [12]. For larger extensions of the phase measurement range a two-wavelength method can be used, because the PTF for a second wavelength is slightly different from the first one. The tuple of the two intensities I_{532} and I_{633} is than used as PTF for phase change evaluations (figure 6). With this technique phase changes of several π radians are possible [9].

4 Optimized Data Acquisition for Flow Field Analysis

Seeding a fluid with tracer particles to measure the velocity field has proven to be a reliable concept, but imaging the tracer particle field is a challenging task in microfluidics due to the usually poor signal to noise ratio on microscopic scales. DynPC microscopy is used to acquire high contrast images of tracer particle fields

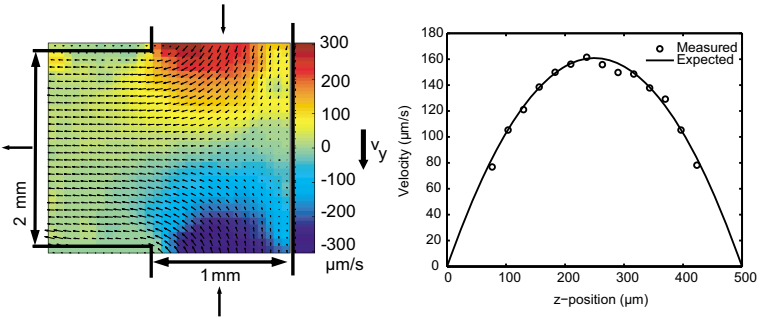

Fig. 7 PIV-measurement of a typical laminar flow field in a T-channel. The y-component of velocity is visualized by the colour coded background. On the right the maximum velocities of a 500 μm height rectangular channel are plotted for different z-levels

with a high signal to noise ratio (see section 3.1). For the demonstration of the capabilities of DynPC microscopy for μPIV measurements we determined the 2D velocity field of a micro-T-mixer, which will be described in section 6. In figure 7 the velocity in the middle layer of the channel with a heigth of 500 μm and width of 1 mm at the inlets and 2 mm at the outlet is shown.

Due of the particular axial response of dynamic phase contrast microscopy as described in 3 it is also possible to realize scanning-2D PIV evaluations. On the right side of figure 7 the maximum flow velocity for different z-positions of a 500 μm high rectangular channel is shown. Because the photorefractive filter unit is a modul mounted between the microscope and the camera all typical methods to obtain 3D velocity informations, like optical sectioning [16] or stereoscopic μ-PIV [14] can be realized with DynPC microscopy.

5 Photorefractive Velocimetry

The basic concept of photorefractive velocimetry is based on the unambiguous dependence of trail length and trail intensity on the object velocity (section 3). Trail length and object velocity correlate linearly and thus enable measurement of the object velocity by determination of its trail length [26]. Measurement of the trail intensity yields an additional, independent value for the object velocity and offers the opportunity of an instant validation of the measured velocity values.

As a first example of application we simulate a laminar tube flow on a spatial light modulator, taking dark amplitude objects as tracer particles. With the previously calibrated system the particle velocities are detected by analyzing their trail lengths. Figure 8 faces the measured particle velocities with the known velocity distribution. The measured values differ less than 1 px / s from the expected ones. A detailed discussion of photorefractive velocimetry, its velocity measurement range, and errors in velocity determination can be found in [26].

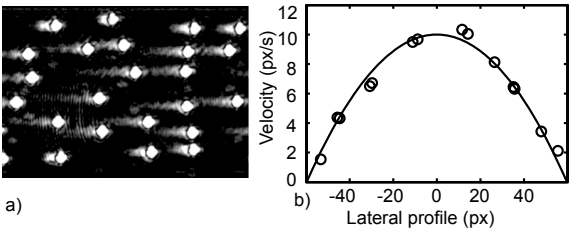

Fig. 8 Laminar tube flow (a) and corresponding velocity profile (b)). The parabolic curve shows the known velocity distribution and the dots indicate the velocities obtained by evaluating the trail lengths

6 Concentration Measurement in Microfluidic Mixing Processes

The phase transfer function of the dynamic phase contrast microscope can be used to quantitatively analyze phase and concentration distributions of mixing processes in fluids and gases [9, 13]. To study micro-mixing of liquids, we constructed a T-shaped mixing channel with a height of 500 μm and a channel width of 1 mm for the input channels and 2 mm for the outflow. After measuring the PTF, we started the phase change measurement and let a NaCl-solution in water (85.5 mmol/l) flow into the channel. These two liquids are barely distinguishable in colour and transparency and show an optical phase difference of approximately 1.5π radians. Because of this large phase difference we implemented a two-wavelength dynamic phase contrast to measure the concentration changes in this channel [9]. In this experiment the liquids were pumped into the channel with a syringe pump with an average flow rate of 36 ml/s, resulting in a laminar flow. Due to the laminar flow, the two liquids do not mix with each other. A diffusive mixing process was introduced by stopping the flow and was analysed by comparing the output intensities with the PTF. Owing to a linear relationship of the refractive index and the concentration C of an aqueous sodium chloride solution for the used wavelengths ($\lambda_1 = 532$ nm and $\lambda_2 = 633$ nm) [24] we can calculate the concentration change ΔC from the optical path length difference OPD. Figure 9 shows the result after the calibration has been applied to the images. The minimum change in the optical path length that we can currently detect with the system is related to $\lambda/40$ which corresponds to a concentration change of $4 \cdot 10^{-6}$

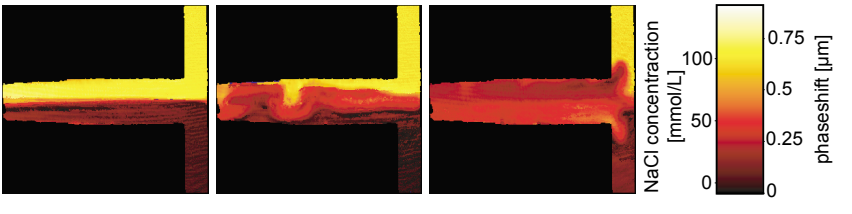

Fig. 9 Measurement of concentration changes due to diffusive mixing of a NaCl solution (85.5 mmol/l) in water. The inflow of the two fluids was stopped after taking the first image. Timespan between the three images is 30 seconds (framerate of the imaging system is 30 fps)

mol/cm^3 for a channel thickness of 500 μm. The maximal concentration change in the unique reconstruction range is up to 10^{-3} mol/cm^3 [9].

7 Summary

Nonlinear dynamic phase contrast microscopy is a phase sensitive method that enables real-time observation of dynamic processes on microscopic scales. In the wide field of microfluidic flow analysis the DynPC microscope can be profitable in three ways. Firstly, tracer particles are highlighted, while any static background is suppressed. This yields optimal image data for subsequent PIV/PTV evaluations. Secondly, if the system time constant is chosen appropriately, trail formation is exhibited and trail lengths and intensities allow for a direct access to flow velocities. Thirdly, micro-mixing processes can be observed without any labelling processes at all. The phase transfer function of the DynPC microscope enables a quantitative, spatially resolved analysis of concentration distributions in real time.

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