Depth Resolution of Dynamic Phase-Contrast Microscopy

M.Eßeling, F. Holtmann, M. Oevermann, M. Wördemann and C. Denz
Institut für Angewandte Physik, Westfälische Wilhelms-Universität Münster,
Corrensstr. 2-4, 48149 Münster, Germany
e-mail: michael.esseling@uni-muenster.de

Abstract: *We present detailed measurements of the particle response function of Dynamic Phase-Contrast (DynPC) Microscopy and its application for microfluidic velocity field measurements.*

Microfluidics is one of the most up-to-date fields of physical research due to the potentials for highly sensitive and efficient medical and chemical analysis [1]. The combination of microfluidic systems with optical functions leads to the highly attractive field of optofluidics [2]. Optical techniques are especially the choice for the measurement of full three-dimensional velocity fields in microfluidic flows. By seeding the flow with micro- or nano particles, micro Particle-Image-Velocimetry (µPIV) can detect the velocity field [3]. Optical sectioning via the depth of field of a microscope objective or stereoscopy are the most used techniques for three-dimensional measurements of micro-flow fields [4]. However, commonly used µPIV algorithms are strongly dependent on sufficient signal-to-noise-ratio (SNR) and intensity stability [5]. A method to improve the SNR and to minimize the error rate of µPIV algorithms is Dynamic Phase-Contrast (DynPC) Microscopy based on a photorefractive novelty filter [6,7]. It suppresses static background and thus highlights dynamics in microfluidic systems [8].

For optical sectioning, it is necessary to know the three-dimensional response of DynPC on a micro particle. Observing transparent particles, the response function of conventional bright field microscopy is asymmetric (figure 1), because light is scattered and focused by the particle. In contrast to conventional microscopy, DynPC shows a symmetric response and higher signal-to noise ratio (figure 2).

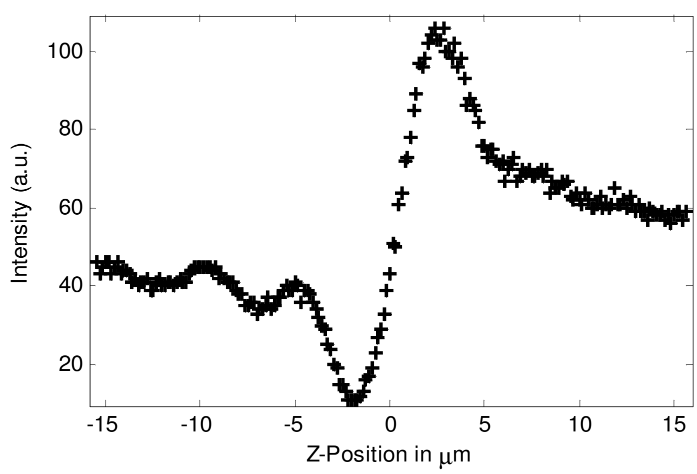

Figure 1: Z-Cut of beads (diameter 1 µm) as seen with partially coherent illumination (λ = 532 nm). The spatial coherence of the laser light is eliminated with a rotating diffuser.

The microscope objective has a NA of 0.45 with a magnification of 20.

In this contribution, we will present a detailed investigation of the particle response function of DynPC Microscopy for typical numerical apertures used in µPIV ranging from 0.45 to 1.4 and compare them to the according results for conventional, partially coherent microscopy, especially in terms of symmetry. Using these results, we will discuss the consequences for the application of DynPC Microscopy in micro-flow 3D-velocity detection. Finally, we will show measurements of microfluidic velocity fields with DynPC Microscopy utilizing its three-dimensional particle response function.

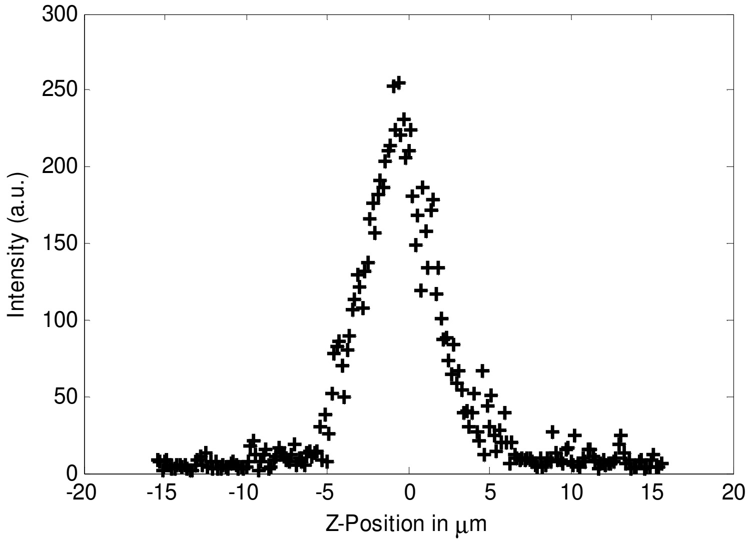

Figure 2: Z-Cut of beads (diameter 1 µm) as seen in the Dynamic Phase-Contrast microscope. Note the symmetry of the response function as compared to the asymmetric response in Fig.1

The microscope objective has a NA of 0.45 with a magnification of 20.

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