

Seeing double and benefiting from it - An easy way to perform 3D particle tracking, sizing and temperature measurements in microfluidics

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Keywords: defocusing, microfluidics, particle tracking velocimetry, thermometry.

ABSTRACT

A new double-refractive imaging method for 3D particle tracking and sizing is presented making use of the birefringent material LiNbO₃. No further modification of a typical microfluidic particle tracking velocimetry setup is needed, which opens up an easy way to 3D flow field measurements. The simultaneous measurement of the particle size enables the analysis of size-dependent phenomena. The lack of optical aberrations also allows to extract more details from other objects than spherical particles, for example pollen grains or other biological specimen. In addition, temperature measurements using lifetime imaging are presented, showing the easy extension of the method for the direct 3D determination of convective heat transfer in microsystems.

1. Introduction

In the last years the demand for reliable 3D particle tracking methods for the characterization of fluidic devices is increasing (Lee et al., 2024; Cierpka & Kähler, 2011), see also the first defocusing particle tracking workshop held at the Lisbon meeting in 2024, which initiated the first Defocus Particle Tracking Challenge (Rossi et al., 2025) with results presented at the Lisbon meeting this year (Rossi et al., 2026). Especially in microfluidics where boundary conditions and external force fields are often only partially known, the experimental characterization is necessary to understand the systems behavior or adapt numerical models (Sachs et al., 2022). The method we want to introduce, has certain advantages over conventional defocusing methods based on pinholes, optical aberrations, defocus or additional astigmatism. The technique is based on the double refraction of a birefringent material and thus called double refractive particle tracking velocimetry (DRPTV). This could be, for example, lithium niobate (LiNbO₃) substrates that are often used for surface acoustic wave excitation. A schematic of the principle setup is shown in Fig. 1.

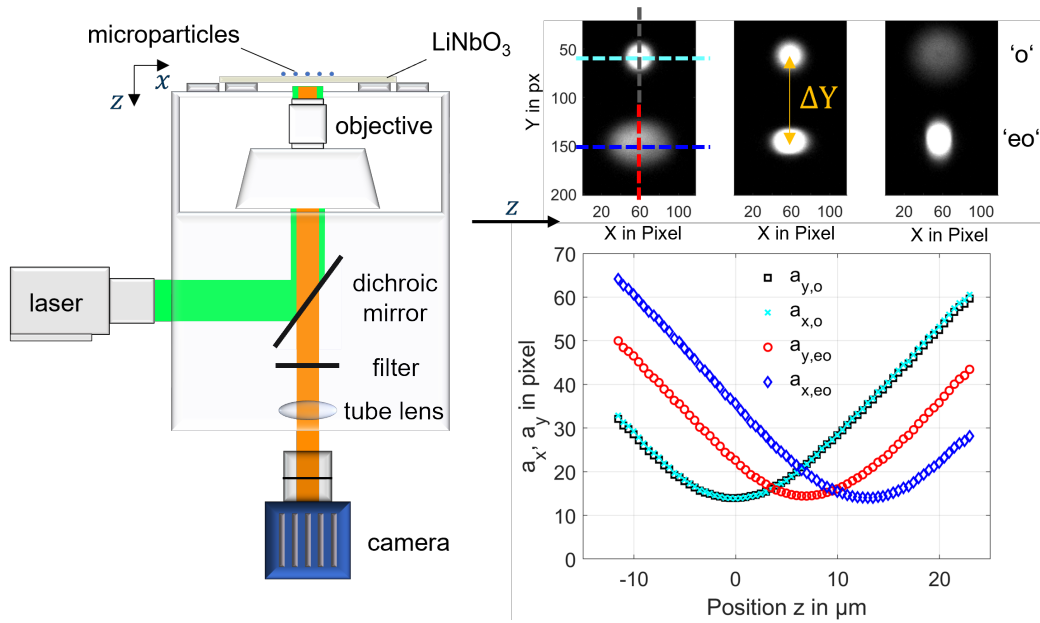


Figure 1. Schematic of the experimental setup with a birefringent plate in the observation path (left) and double images for different depth positions (top right). Particle image extensions a in x - and y -direction for different (axial) z -positions (bottom right).

The LiNbO_3 causes light scattered from a particle in the fluid split into an ordinary ‘o’ and extraordinary ‘eo’ beam. Their different refractive indices in the material yields to a bifocal imaging with an axial shift $\Delta z_{x,y} = F_{o_{x,y}} - F_{eo_{x,y}}$ of the two focal planes in the x - and y -direction, respectively. A particle is now imaged twice on the camera sensor within a constant distance ΔY due to a walk-off angle of the eo-beam in the substrate. In addition, each particle image shows different blur depending on the actual particle position relative to the respective focal plane. Analyzing the double images, a calibration function can be derived that is proportional to the difference of the particle image diameters (a_o, a_{eo}) squared $z = f(a_o^2 - a_{eo}^2)$ (König & Cierpka, 2024). One of the main advantages is, that this relation is independent from the physical particle size. This allows to use multi-disperse particle solutions in comparison to the classical defocusing methods (Barnkob et al., 2021). In addition, knowing its 3D position, the particle size can be determined simultaneously (König & Cierpka, 2024).

2. Experiments

To prove the applicability of the new method two different experiments were performed. The experimental setup is schematically depicted in Fig. 2 on the left, whereas the two different used microchannels are presented in the same figure in the middle and on the right.

To show the applicability for particle sizing, flow measurements were performed in a Y-shaped microchannel made of polydimethylsiloxane (PDMS) with a rectangular cross section of $w \times h =$

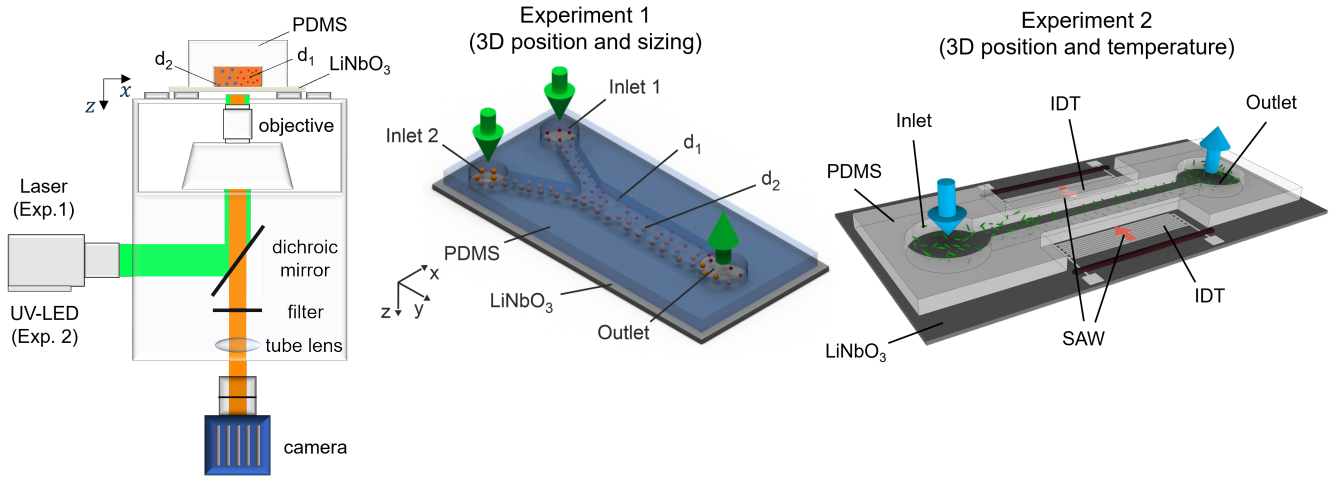


Figure 2. Schematic of the experimental setups. For the additional temperature measurements the laser was replaced by a UV-LED to excite the color dependent dye in the particles (left). For experiment 1 (3D position and sizing) the Y-shaped PDMS channel featuring a birefringent plate as channel bottom is shown with the inlets for the suspension with particles of different sizes $d_1 = 1.14 \mu\text{m}$ and $d_2 = 2.47 \mu\text{m}$ (middle). The temperature measurements were performed in an acoustically excited microchannel where surface acoustic waves (SAW) were initiated by two opposed inter digital transducers (ITD) (right).

$500 \times 185 \mu\text{m}^2$ in the joint part. Two suspensions with different particle sizes in each inlet of diameter $d_1 = 1.14 \mu\text{m}$ and $d_2 = 2.47 \mu\text{m}$ were used at equal flow rates of $2.5 \mu\text{l}/\text{min}$. As channel bottom, a 128° rotated Y-cut LiNbO_3 substrate of $500 \mu\text{m}$ thickness was used and served as the birefringent plate. It is important to note that the birefringent plate as part of the microchannel is not mandatory for the measurement method in general. The birefringent plate could be placed independently of the microchannel, e.g., positioned below in the optical path. Apart from the magnification and numerical aperture of the microscopic setup, the calibration function depends on the strength of the birefringence, i.e. the material chosen, and the thickness of the plate. The sensitivity can thus be adjusted to match the desired measurement volume depth and the particles used.

Due to the small diffusion coefficients ($O(10^{-13} \text{ m}^2/\text{s})$) of the particles this results in a co-laminar fluid flow of both suspensions in the straight part of the microchannel with corresponding particle distributions on either side. This give access to experimental ground truth. The fluorescently labeled polystyrol particles were illuminated independently of the particle size, the laminar flow Hagen-Poiseuille-like velocity distribution was measured successfully applying a nearest neighbor particle tracking algorithm after 3D position determination. The velocity profiles approximately 3 mm after the junction are shown in Fig. 3 (left). The uncertainty was $\sigma_{u,xy}/\bar{u} = 0.43\%$ and $\sigma_{u,z}/\bar{u} = 1.46\%$ for the in-plane and out-of-plane directions, respectively, with $\bar{u}$ being the mean streamwise velocity.

The particle size can be discriminated by the sum of the area of the particle images ($A_{\text{sum}} = A_o + A_{\text{eo}}$) for known depth position (König & Cierpka, 2024). Alternatively, also machine learning

methods could be used (Ratz et al., 2023). The probability density function of the cross-sectional particle distribution is shown in Fig. 3 on the right side. As can be seen, the two streams could be clearly distinguished. A final precision of 98.6% was achieved discriminating the spherical particles used.

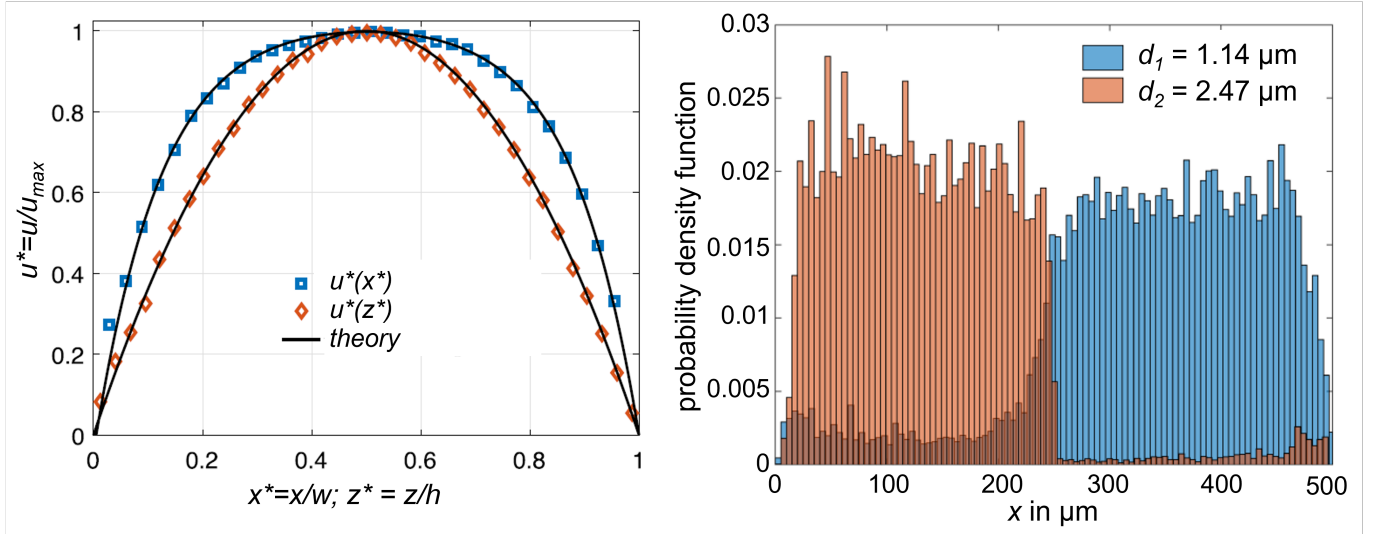


Figure 3. Measured centerline profiles of the streamwise velocity over channel width and height in comparison with theory (left). Cross-sectional particle distribution over channel width (left).

In the case of energy input by external fields, as typical for acoustofluidics used for particle and fluid manipulation (Sachs et al., 2022), the fluid might heat up, especially when small and/or stagnant fluid volumes are under investigation (Weser et al., 2022).

DRPTV can easily be extended to also measure temperature. The light source was simply replaced by a UV-LED (Solis-365C, Thorlabs). The experiments were performed in an acoustically excited microchannel initially used for particle separation (Sachs et al., 2022, 2025). A volume flow rate of $1.5 \mu\text{l}/\text{min}$ was applied in the system. A one-dimensional sSAW is excited by two opposing interdigital transducers (IDTs) deposited on a 128° rotated Y-cut lithium niobate substrate (LiNbO_3). Two different SAW-wavelength were used ($\lambda_{\text{SAW}} = 20$ and $90 \mu\text{m}$) and the aperture (length) of the IDTs was 2 mm in total. In between both IDTs, a straight micro channel made of PDMS with a cross-section of $500 \times 500 \mu\text{m}^2$ was positioned. The DRPTV method was extended using temperature sensitive particles (PMMA doted with temperature sensitive phosphorescent dye europium (III) thenoyltrifluoroacetate (Eutta) 360/615 nm, $d_3 = 10 \mu\text{m}$, Surflay Nanotec GmbH) (Massing et al., 2018). In principle both experiments, the sizing and temperature measurements can be done simultaneously, however only one particle size for the temperature measurements were available and the timing schemes need to be adjusted. The dye in the particles is excited by the UV-LED using a power between 20 and 100 mW and the lifetime of the luminescence signal is measured by

comparing two successive frames of the camera. As the luminescence decay is independent from the actual imaging, the use of the same chip with birefringent bottom is not necessary and a much simpler chamber where a constant temperature can be adjusted was used. Special care has to be given to photo bleaching of the particles by the calibration procedure. For details on the calibration and setup the interested reader is referred to Deng et al. (2022) and Weser et al. (2022). For individual particles, the mean standard deviation was below 0.65 K within a temperature range from 20 to 60°C.

In acoustofluidics two forces act on the particles. Namely the acoustic radiation force that moves particles directly, and the streaming force that acts on particles due to viscous drag. As the acoustic radiation force scales with the particle volume $\sim d^3$ and the drag force only with $\sim d$. Thus, the particle trajectory for the comparably large temperature sensors made of PMMA is mainly governed by the acoustic radiation force.

In order to reliably measure the velocity field, additional measurement with particles with a size of only $d_4 = 450$ nm were performed. As already mentioned the calibration function for the depth position is independent of the particle size and therefore holds for both particle sizes. The IDTs were driven with about 100 mW electrical power each. The acoustic streaming induced cross-sectional flow can be seen on the left side for both wavelength. To determine the Eulerian velocity fields averaging of the Lagrangian velocity vectors over voxels with a lateral size (y, z) of $20 \mu\text{m}$ and a stream-wise length (x) of $800 \mu\text{m}$ with Gaussian distance weighting was performed. The final velocity fields are shown in Fig. 4 on the left side. At $\lambda_{\text{SAW}} = 20 \mu\text{m}$ two vortex roles can be seen, spanning the whole cross section. With larger wavelength, the number of roles increase, which is beneficial for the application for particle separation as mixing over the whole cross-section is prevented (Sachs et al., 2025).

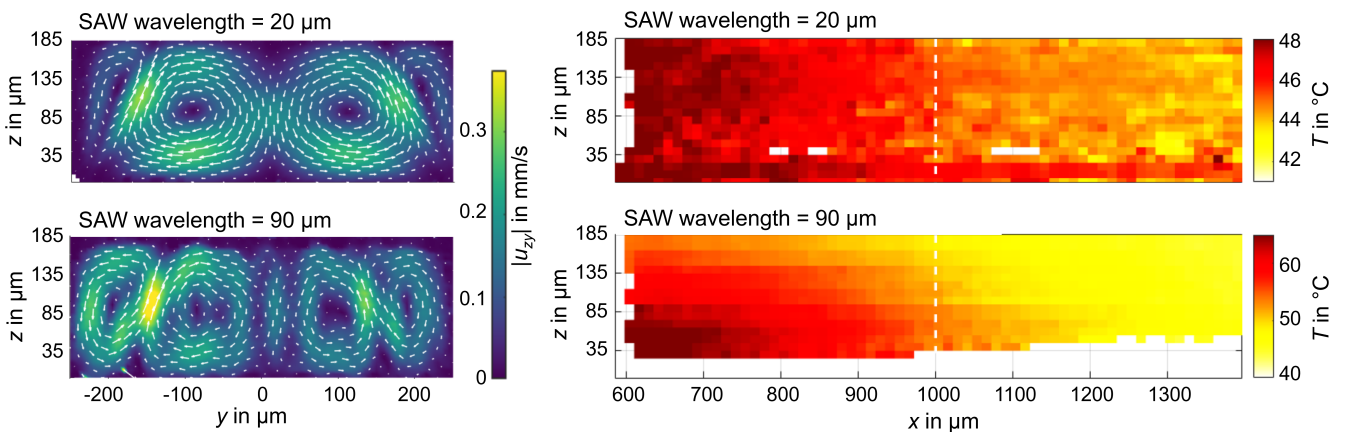


Figure 4. Cross-sectional velocity magnitude for two different SAW wavelengths showing acoustic streaming (left) and temperature distributions in stream-wise direction in the mid-plane of the channel (right). The end of the IDT is indicated with the white line.

The 3D temperature distribution was measured using the larger PMMA particles. The individual

temperature values were again Gaussian averaged for voxel sizes of $25 \times 25 \times 60 \mu\text{m}^3$. The resulting temperature field is shown in Fig. 4 on the right side. The larger averaging in the y -direction was necessary, as the acoustic radiation force causes a non-homogeneous distribution of the large temperature sensitive particles in the channel. The end of the IDT is marked by the white line. It can be clearly seen for both wavelengths that the temperature in the actuated region is much higher. Values of up to 48°C for $\lambda_{\text{SAW}} = 20 \mu\text{m}$ and up to 65°C for $\lambda_{\text{SAW}} = 90 \mu\text{m}$ were observed close to the channel bottom where the acoustic energy of the SAW is introduced into the fluid. Right after the region where the SAW-field is present, the temperature drops fast for both cases. Further measurements for different electrical power showed a nearly linear increase of the mean temperature with applied power. For life science applications this is important as elevated temperatures may be harmful for biological specimens.

3. Conclusions and Outlook

The new double-refractive imaging method for particle tracking and sizing was presented making use of the birefringent material LiNbO_3 . No further modification of the setup is needed, which opens up 3D flow field measurement for many researchers. The simultaneous measurement of particle size enables the analysis of size-dependent phenomena. The lack of optical aberrations also allows to extract more details from other objects than spherical particles, for example pollen grains or other biological specimen. In addition, temperature measurements were presented, using lifetime imaging showing the easy extension of the method for the direct 3D determination of convective heat transfer in microsystems.

Beyond the particle image size for the 3D localization, the double images yield additional information by exploiting chromatic dispersion and polarization effects of the birefringent plate. The distance between the double images (ΔY), which is governed by the emission wavelength of the light emitted by the particles can be used for example for temperature measurements using thermochromic liquid crystals (Segura et al., 2015) or distinguish different fluorescent particles. Using a birefringent plate in a standard microscope would therefore allow for a cheap extension towards multimodal imaging and is expected to become more relevant for the ever increasing demand for the reliable characterization of microfluidic systems.

Acknowledgements

The authors gratefully acknowledge Tim Essebier for his valuable support in the lab. Funding by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) within the priority program PP2045 “MehrDimPart” (project number 444806275) is gratefully acknowledged.

Nomenclature

X, Y	Image coordinates [Pixel]
A	Particle Image Area [Pixel]
x, y, z	Physical coordinates [μm]
a	Particle Image Size [Pixel]
I	Particle Image Intensity [counts]

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