

3D Measurements of Transient Microscopic Phenomena using a Double-Helix Point Spread Function and a Convolutional Neural Network

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Keywords: 3D Particle Tracking Velocimetry, Microfluidics, Fluorescence Localization Microscopy, Cleaning-in-place, Machine Learning, CNN Image Processing

ABSTRACT

Microscopic multiphase flows play an important role in a wide variety of applications such as process engineering (e. g. separation, transport or cleaning processes) and biomedicine (e. g. mixing in droplet microreactors). We investigate film flows occurring at spray cleaning as well as the spray-induced cleaning of soil from surfaces by applying High-Speed 3D Fluorescence Localization Microscopy based on the Double-Helix Point Spread Function with frame rates up to 1 kHz. This single-camera technique offers a high axial measurement range of approximately 1000 μm at a comparatively low localization uncertainty. We present measured near-wall film flow profiles with a resolution of up to 7.3 μm that were measured with a single access and subsequently determine the wall-shear stress. In order to investigate the transient process of spray-induced soil removal from a surface, we apply convolutional neural networks to locate particles at high particle densities and low signal-to-noise ratios. The results pave the way for 3D measurements of transient microscopic processes with a high temporal and spatial resolution.

1. Introduction

Effective cleaning of industrial equipment is critically linked to product quality, process stability, and resource efficiency. To ensure reproducible performance, many sectors rely on automated cleaning-in-place (CIP) systems, where atomizing nozzles generate sprays (Kalantari (2008)) that strike surfaces and form gravity-driven liquid films (Fuchs et al. (2015)). The mechanical action of both droplet impact and the subsequent film flow, particularly the wall shear stress, largely determines cleaning effectiveness.

Despite their importance, the dynamics of these thin films remain not fully understood (Breitenbach et al. (2018)). Film behavior is governed by gravitational, viscous, and capillary forces, and

is further complicated by interactions between polydisperse droplets and the developing surface film. Prior studies have shown that parameters such as wall shear stress and viscous sublayer thickness correlate strongly with cleaning performance (Fuchs et al. (2015)), yet obtaining spatially resolved velocity profiles (Bashkatov et al. (2025); Bürkle et al. (2025)), especially near the wall, remains challenging.

Advances in 3D Fluorescence Localization Microscopy (Cierpka and Kähler (2012); König and Cierpka (2024); Pavani et al. (2009)) have enabled the measurement of microscale flows with one single optical access. Nonetheless, achieving a sufficiently high temporal resolution for comparatively fast flows (~ 30 mm/s) is still challenging for 3D localization microscopy. The main reason is that 3D Localization Microscopy is usually realized via fluorescence particles with relatively low emission intensity since elastically scattered light does in many cases not yield a sufficiently low axial localization uncertainty (Buchmann et al. (2014)). Furthermore, since the particle density is usually much lower than in macroscopic Particle Imaging Velocimetry, the measurement of transient processes (Maestri et al. (2025)) is challenging.

In this conference contribution, we present High-Speed Fluorescence 3D Localization Microscopy (Yang et al. (2026); Bilsing et al. (2025); Zhang et al. (2026)) based on the Double-Helix Point Spread Function (Fig. 1), which we term Double-Helix Particle Tracking Velocimetry (DH-PTV). We show measured near-wall profiles of film flows that are induced by an atomizing nozzle and estimate the wall shear stress from the measured profiles. Additionally, we present proof-of-principle measurements of the transient process of spray-induced soil removal from a glass plate. In order to improve the resolution of the transient process measurement, we employ convolutional neural networks (Tasmany et al. (2026)) for image processing at high emitter densities and relatively low signal-to-noise ratio.

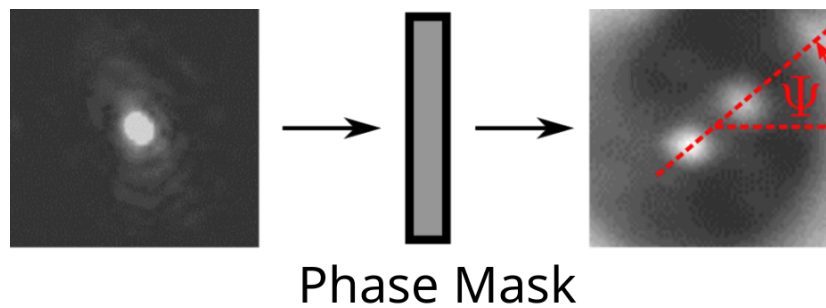


Figure 1. Basic principle of position measurement using a Double-Helix Point Spread Function (DH-PSF). In conventional imaging, a fluorescent particle appears as a single point. With the DH-PSF, the particle is imaged as two bright spots whose midpoint gives the lateral position (x, y), while their orientation angle ψ is approximately proportional to the axial position (z). A spiral phase mask in the pupil plane of the optical system modulates the light spatially to introduce the DH-PSF.

2. Experimental Methods and Results

Fig. 2 shows a sketch of the inverse fluorescent microscope used for the film flow experiments with DH-PTV. A DPSS laser (cw, $\lambda = 532$ nm, $P_{\text{opt}} = 1$ W, RLTMGL-532-1W by Roithner Lasertechnik GmbH) excites fluorescent particles (PS-FluoRot-10 by Microparticles GmbH, excitation/emission wavelength: 530 nm/607 nm) in the measurement volume. The fluorescent light is separated via a dichroic mirror from the excitation light. A lithographically manufactured phase mask is placed in the pupil plane (resp. fourier plane) of the system and introduces the Double-Helix Point Spread Function. The camera (EoSens1.1MCX12-CM by Mikrotron GmbH) enables frame rates of up to approximately 5.2 kHz in this configuration.

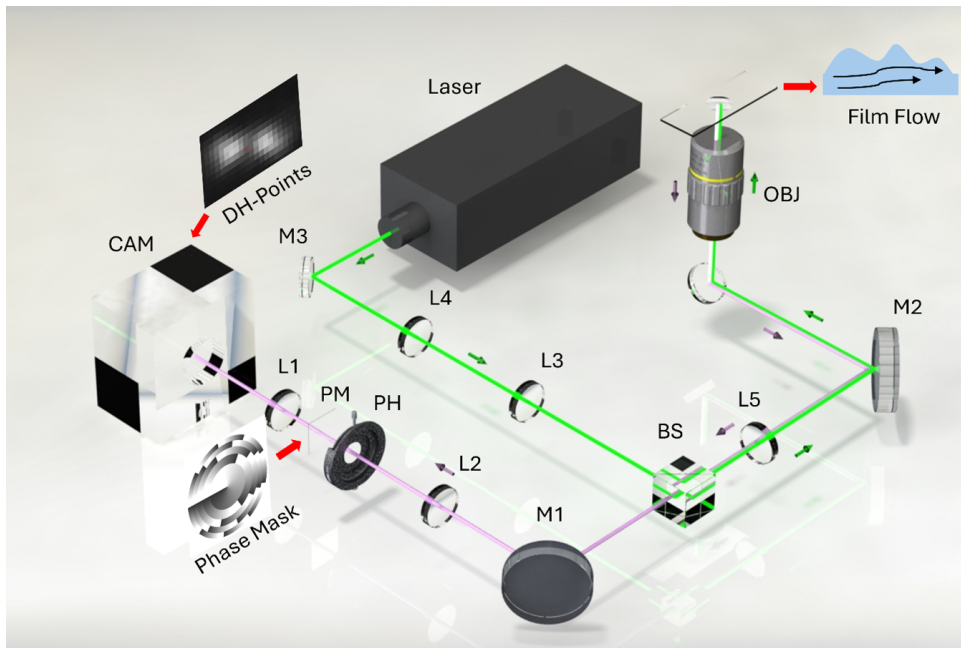


Figure 2. Overview of experimental setup for microscopic film flow measurements. Green rays: excitation light. Purple rays: Fluorescence light. OBJ: microscope objective, CAM: CMOS camera sensor, M1-M3: mirrors; L1-L5: lenses, PH: Iris Aperture, BS: beam splitter cube, PM: phase mask. (Yang et al. (2026))

Fig. 3 shows the setup used for investigating the film flows that occur at spray cleaning. The spray cleaning setup circulates water containing fluorescent tracer particles driven by a centrifugal pump (EBARA Matrix 3-7 T). Before reaching the nozzle, the flow passes through a calming section that ensures a stable and uniform inlet condition. The fluid is then discharged through a flat fan nozzle toward a horizontal glass plate, where impacting droplets spread into a film. This film flows radially outward from the impact region. The glass plate serves both as the target surface and as an optical interface that allows measurements from below while keeping the optical equipment protected from water. The DH-PTV measurement system is mounted underneath the plate and can be traversed laterally to record local film-flow velocities at multiple radial positions and operating pressures. A distance of 150 mm between the nozzle and the glass plate is employed

for all experiments.

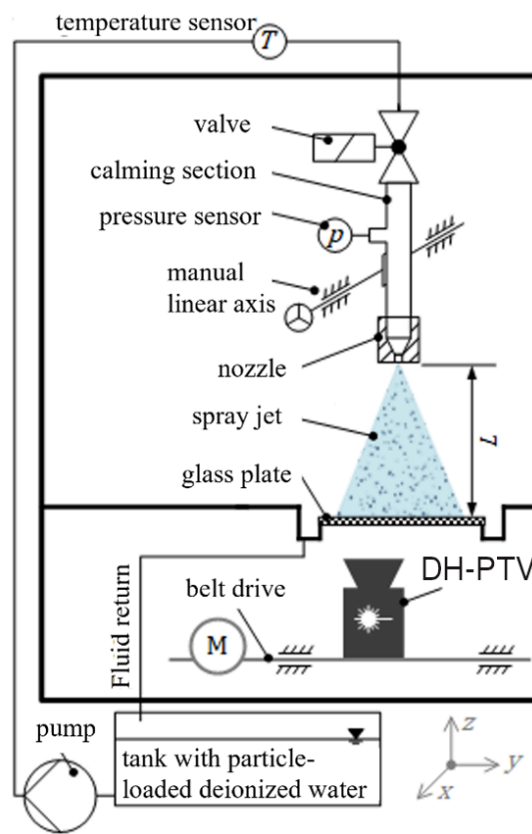


Figure 3. Schematic of the experimental arrangement used to study film flow and spray cleaning. Deionized water seeded with tracer particles passes through a nozzle to generate a spray directed onto a glass plate. The DH-PSF system positioned beneath the plate captures the film flow field, supported by sensors and adjustable axes for precise alignment and operation. (Yang et al. (2026))

The near-wall film flow was measured for two different nozzle pressures (1 bar and 3 bar) by capturing 40,000 images for each position (Fig. 4). Fig. 5 shows the results. It can be seen that the measured flow profiles exhibit a significant dependence on the radial position and the pressure. The spatial resolution along the axial direction is $7.3\ \mu\text{m}$ for the flow profile. By determining the linear slope near the glass surface the wall shear stress can be estimated (Fig. 6). Accordingly, the wall shear stress decreases with a tendency of about r^{-1} . This is in agreement with an estimation based on constant film thickness along the radial direction and a linear flow velocity profile (Yang et al. (2026)).

In order to investigate the spray cleaning process, we apply a layer of caramel syrup (Grafschafter Karamell, layer thickness between $500\ \mu\text{m}$ and $1000\ \mu\text{m}$) seeded with Fluorescence particles (PS-42 from *Radiant Color NV*) as a model soil on the glass plate. A refractive index of 1.4965 of the caramel syrup was measured with a refractometer. The movement of the particles was measured with the setup described in Fig. 2 at frames rates up to 1 kHz. The boundary conditions for the

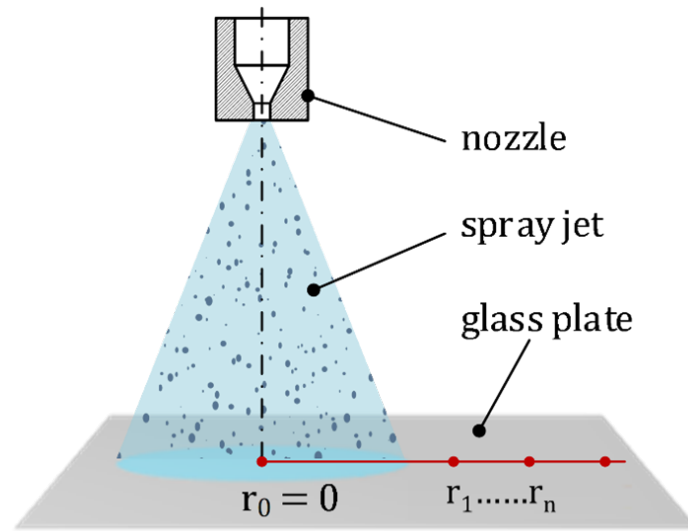


Figure 4. Sketch of the spray cleaning process and the distance between the jet impact point and the measurement position r . The measurement positions were aligned with the major axis of the nozzle and were located outside the direct drop impact zone. (Yang et al. (2026))

measurement of this transient process are significantly different in contrast to the film flow (Fig. 5) since the cleaning of the glass plate is time-limited and can be as short as 2 s in this configuration. Therefore, the measurement time can not be chosen arbitrarily long anymore as for the film flow. Fig. 7 shows a proof-of-concept measurement of the caramel syrup thickness over time.

In order to significantly improve the temporal and spatial resolution despite these challenges, we increase the particle density and employ convolutional neural networks to enhance the localization performance at higher particle densities. The network structure and the general approach is adopted from Nehme et al. (2020). The neural network is trained via simulation of the physics-based point spread function. We compare the results to the non-learning based particle localization algorithm described in Crocker and Grier (1996), which is implemented in the software Grüter (2025). Fig. 8 shows a comparison of the particle localizations for one frame. Fig. 9 shows the number of detected particles for different tracer particle concentrations in the caramel syrup. It can be seen that the neural networks improves the localization count approximately by a factor of 2.

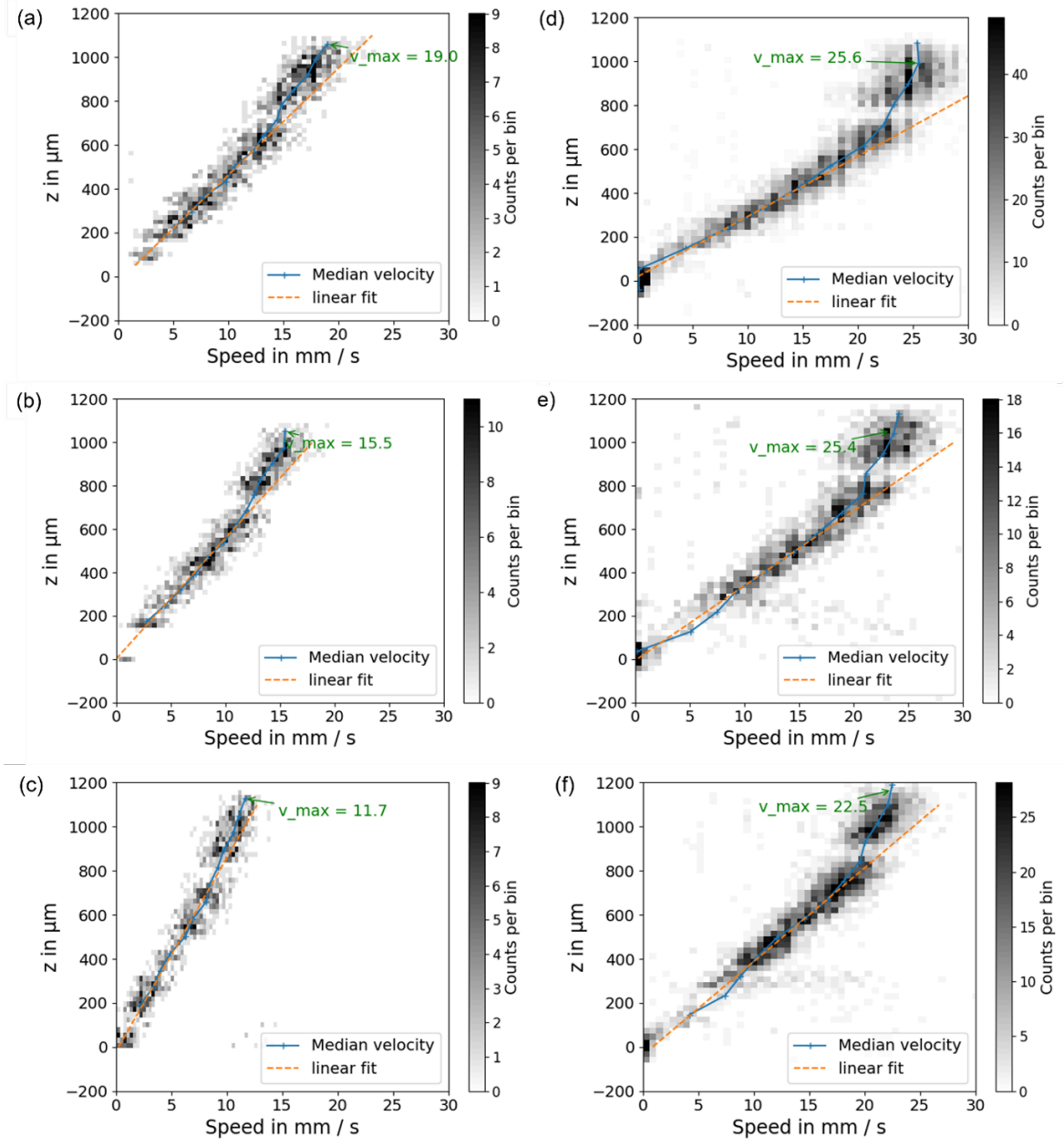


Figure 5. Panels (a)–(f) display depth-resolved velocity profiles at the radial positions $r_1 = 192$ mm, $r_2 = 216$ mm, $r_3 = 240$ mm for two pressure settings. Subfigures (a)–(c) present measurements at 1 bar water pressure, while (d)–(f) provide the corresponding profiles at 3 bar. The grey scale indicates the probability density of detected particles, and the reported velocities represent only the horizontal component, as out-of-plane motion is negligible in the measurement domain. The blue curves show the median-binned velocity profiles, and the orange dashed lines represent linear regressions applied to the near-wall region, whose slopes are used to determine the wall shear stress. (Yang et al. (2026))

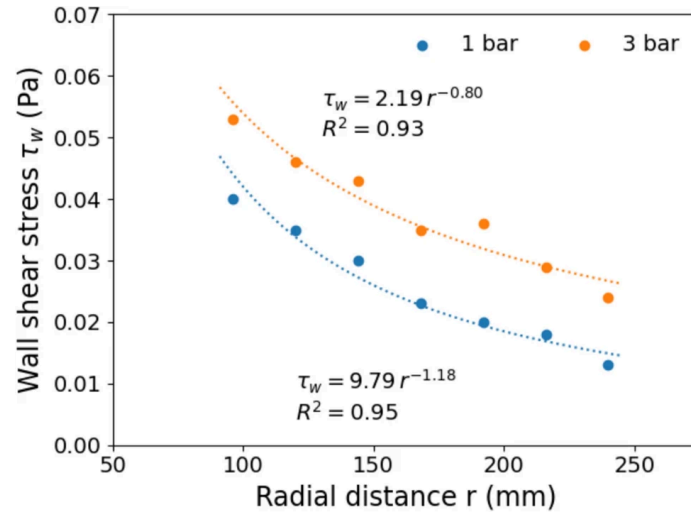
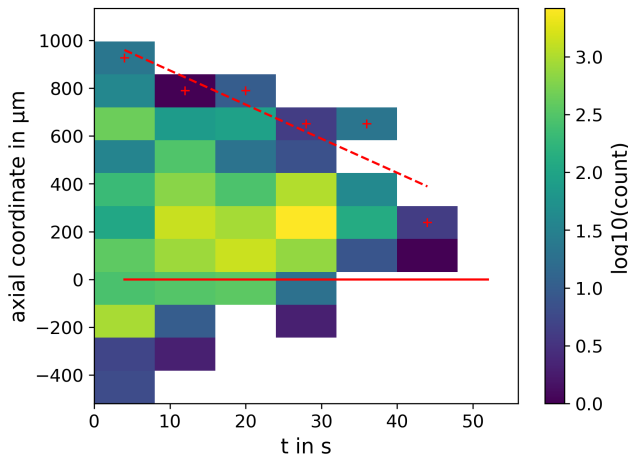
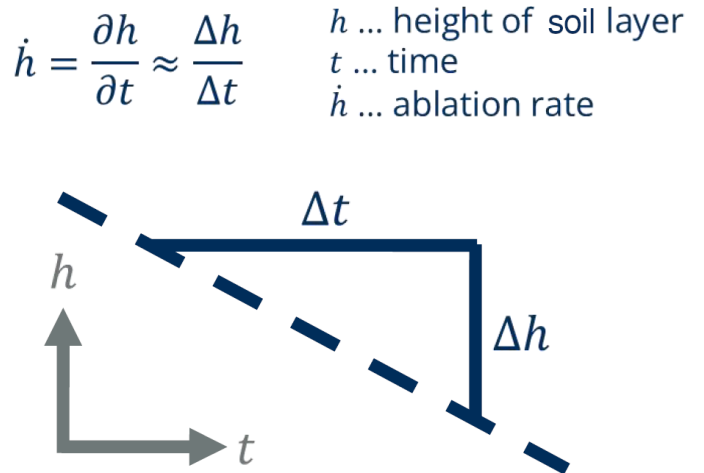


Figure 6. The experimentally measured wall shear stress varies with radial distance from the jet center at a nozzle-to-surface spacing of $L = 150$ mm, under two inlet pressure conditions (1 bar and 3 bar). The dots denote measurement results, whereas the dotted line illustrates the model found by regression. R^2 is the coefficient of determination. R^2 indicates how well the data fits to the model and ranges from 0 to 1, where 1 corresponds to perfect agreement. (Yang et al. (2026))

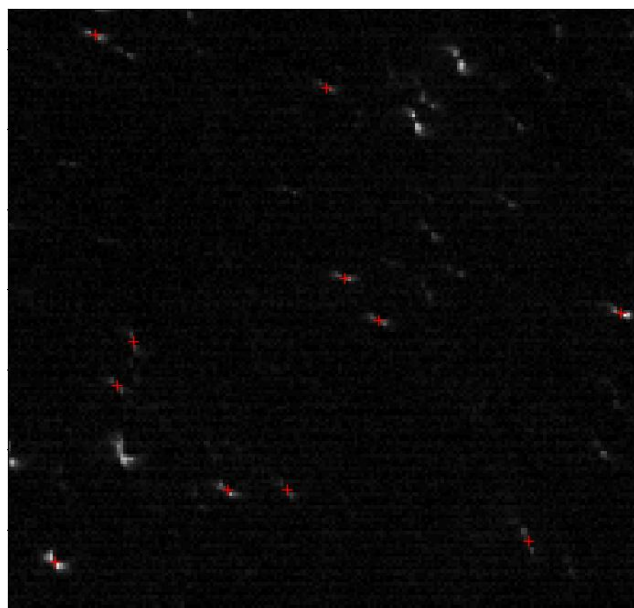


(a) Proof-of-principle measurement of the caramel syrup layer thickness over time. Each interrogation window shows the number of particles that were detected at the corresponding height and time in a log scale. White interrogation windows indicate that no particle was detected. The red solid line indicates the approximate position of the glass plate.

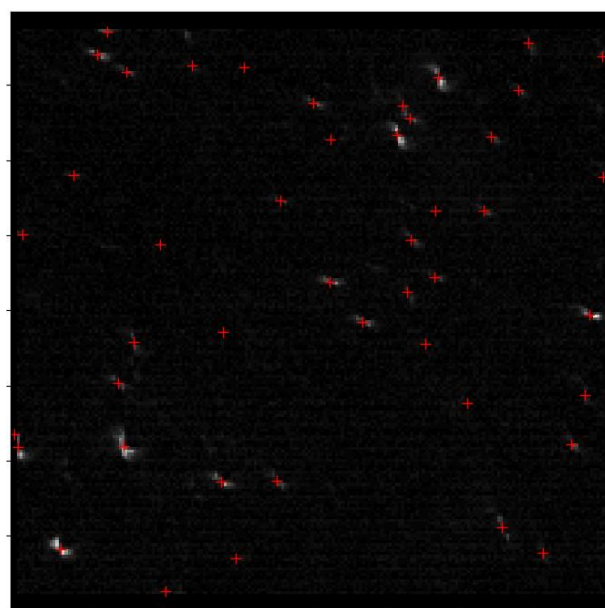


(b) Sketch for illustrating the calculation of the ablation rate from Fig. 7a. A regression line is calculated from the top points of the layer at each time step (denoted with red crosses in Fig. 7a). The slope of this line denotes the ablation rate in $\mu\text{m/s}$.

Figure 7. Measurement of caramel syrup cleaning and sketch describing how the result was evaluated.



(a) 11 located particles with algorithm from Crocker and Grier (1996) implemented in PtvPy Grüter (2025)



(b) 45 located particles with convolutional neural network. The network structure and the training is adopted from Nehme et al. (2020).

Figure 8. Comparison of particle localization with non-learning based technique (a) and neural networks (b).

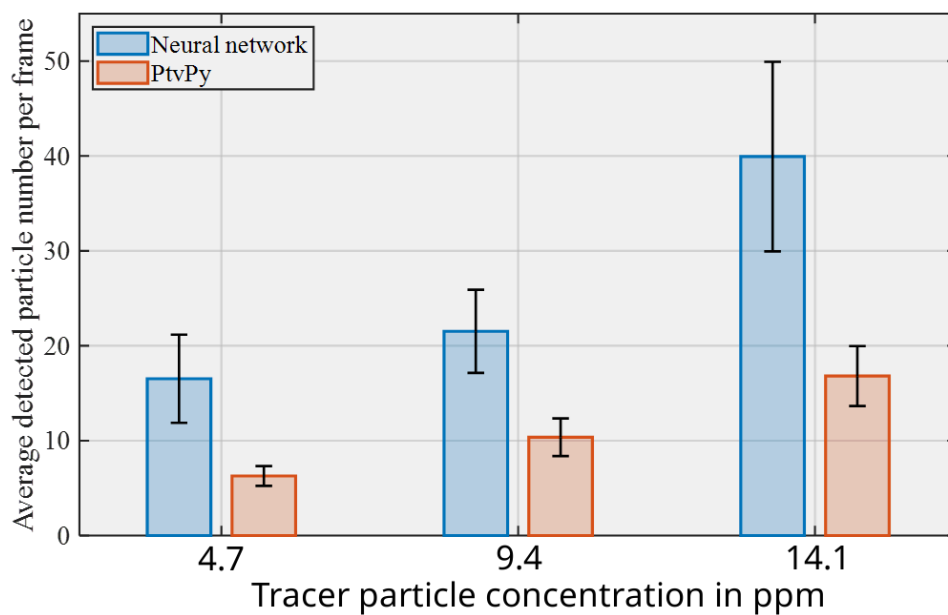


Figure 9. Number of detected particles for different seeding concentrations (related to mass ratio) in the caramel syrup. It can be seen that the neural network consistently detects more particles per frame than the non-learning based algorithm (*PtvPy*). Tracer particles PS-42 from *Radiant Color NV* were used.

3. Summary

We show high-speed microscopic flow measurements using 3D Localization Fluorescence Microscopy with a Double-Helix Point Spread Function, reaching frame rates up to 1 kHz. The approach is applied to spray-induced film flows. The high spatial resolution of the film flow profiles of up to $7.3\text{ }\mu\text{m}$ enabled the estimation of the wall shear stress. Furthermore, the removal of a caramel syrup layer at spray cleaning is investigated. In order to reduce the measurement time and to measure transient processes, convolutional neural networks are employed that are capable to evaluate images with significantly higher particle density. The results can pave the way to new insights into internal film flow dynamics, spray cleaning processes and transient phenomena.

Acknowledgements

The IGF research project 22492 BR from the research association IVLV e. V. is supported by the Federal Ministry of Economic Affairs and Energy through the German Center for Aerospace e. V. (DLR) as part of the programme for promoting industrial cooperative research (IGF) on the basis of a decision by the German Bundestag. Furthermore, this work is partially supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - 459505672.

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