Physikalisch-Technische Bundesanstalt
National Metrology Institute

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Multi-Dimensional Imaging of Anisotropic Particles in Flow Increases Cell Counting Accuracy

Motivation & Goal

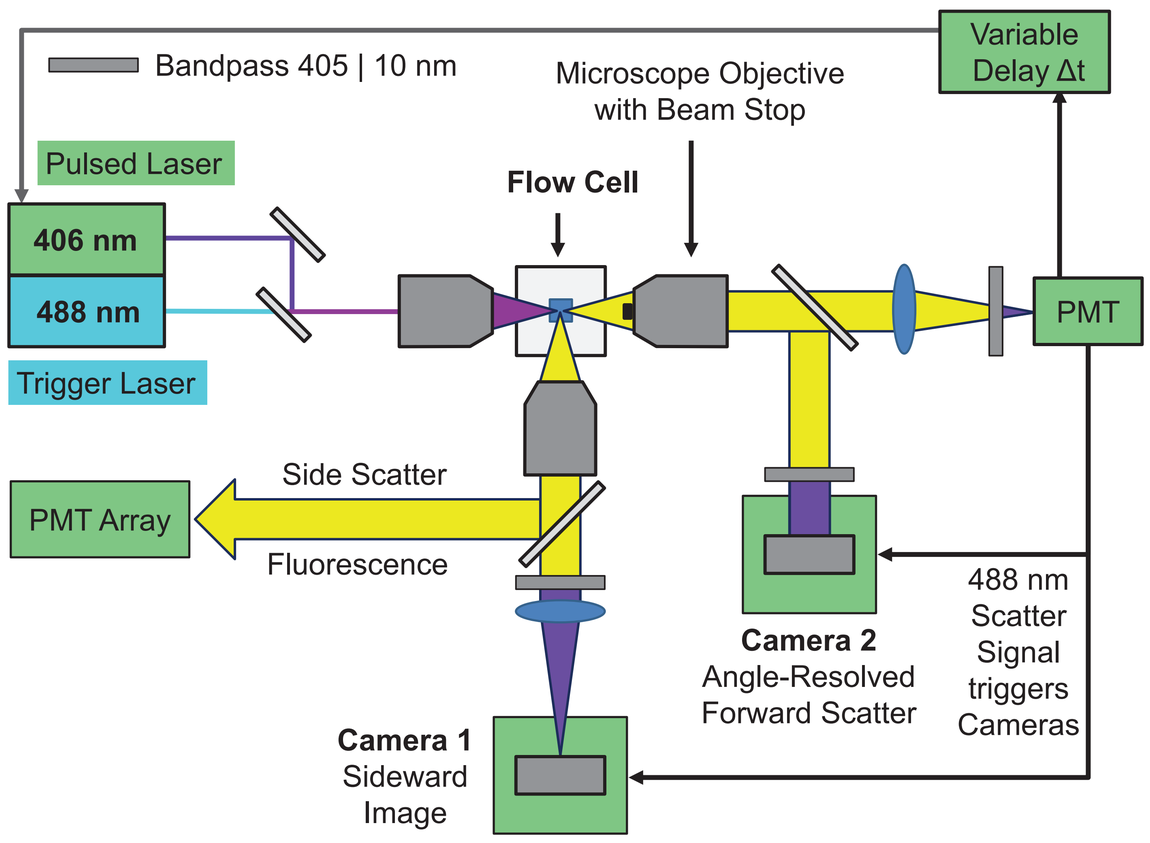
Laser flow cytometry is routinely used in laboratory medicine to count blood cells to diagnose the immune status and detect diseases like leukaemia. However, counting accuracy is limited by:

- coincidences of two or more cells
- cell agglomerates
- different cell orientations
- dynamic changes of the cell shape

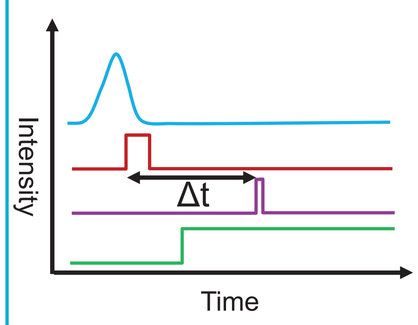
We develop a reference flow cytometer to overcome these limitations and increase counting accuracy by multi-dimensional imaging and angle-resolved scattering capabilities. We facilitate personalized medicine – ultimately saving lives.

Experimental Setup

- microscopic imaging of sample stream at flow speeds of m/s requires exposure times of < 1 µs
→ realization: cw 488 nm scatter signal from chosen photomultiplier tube (PMT) triggers 406 nm laser pulse and CMOS cameras
- camera 1: image in sideward direction
- camera 2: angle-resolved forward scatter (‘Angle-Resolved FSC’, ‘imaging to infinity’)
→ beam stop blocks laser within 3° solid angle
- **samples**: anisotropic silica-hybrid microparticles in ultrapure water
- through hydrodynamic focusing cells pass elliptical laser focuses (~20 µm) one by one

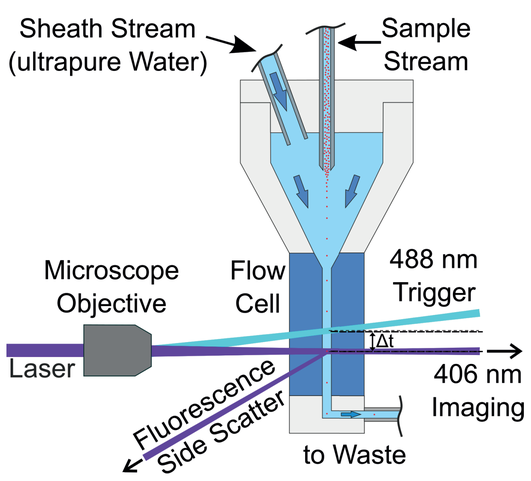


Trigger Mechanism



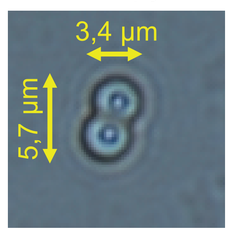
(1) FSC Signal (3-15) µs
(2) Camera Trigger (1 µs)
(3) Laser Gate (0.1-1.0 µs)
(4) Exposure active (>30 µs)
Δt : Variable Delay

Hydrodynamic Focusing

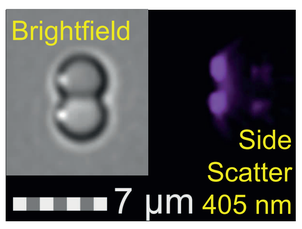


Results: Dumbbells

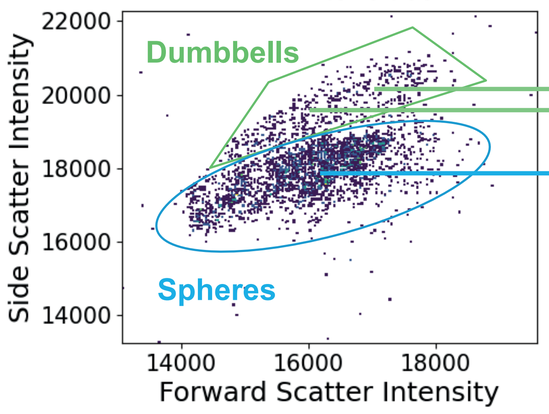
Microscopy 40x



Amnis ImageStream[x]



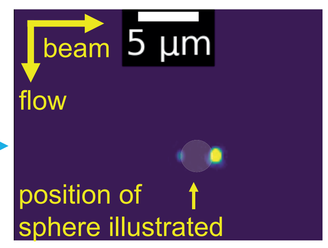
PMT Intensities



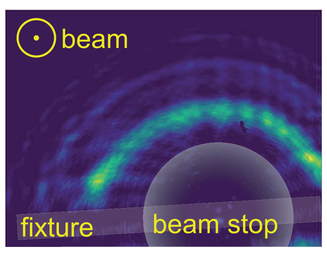
Sample separates into dumbbells and spherical objects (‚spheres‘)

Sphere

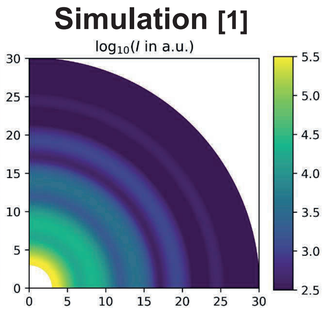
Sideward Image



Angle-Resolved FSC



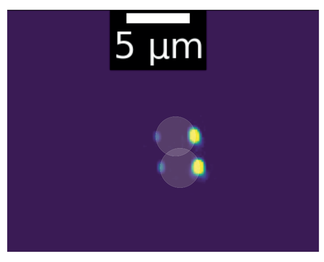
Simulation [1]

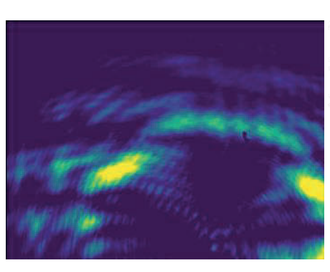


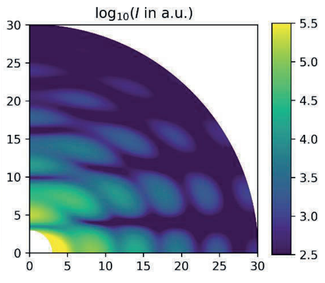
1 bright + 1 dim spot possibly due to scatter from particle surface

- circular pattern in experiment and simulation
- mini interference structures due to different phenomena, e.g. reflections from other optics

Dumbbell tilted by 90° to beam



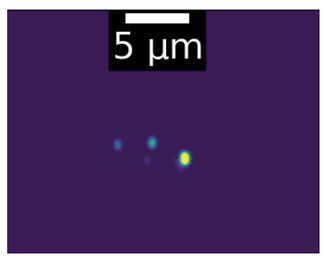


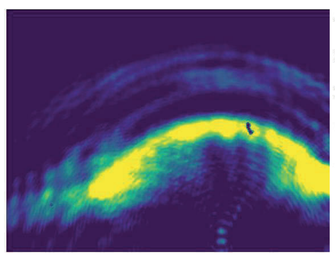


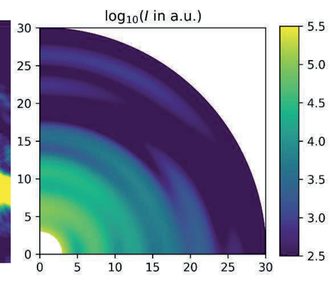
2 bright and 2 dim horizontally dispersed spots indicate dumbbell tilted by 90° to beam

- similar horizontal interference pattern in experiment and simulation
- orientation of pattern fits to orientation of particle in sideward image

Dumbbell tilted by 165° to beam





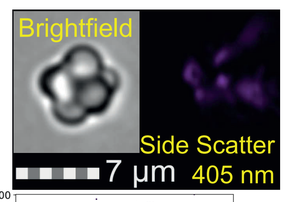


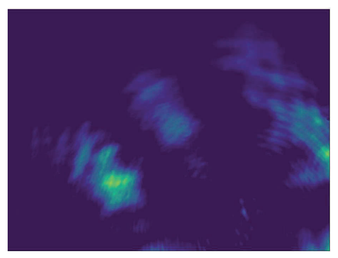
4 spots may be caused by tilted dumbbell or coincidence of 2 spheres

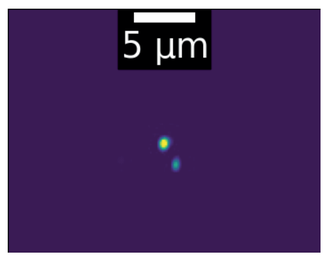
- distorted circular pattern correlates with tilted dumbbell at 165° (beam axis ≙ 0°)

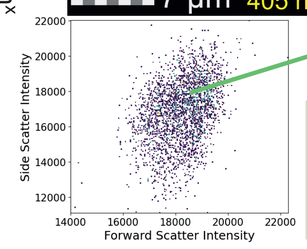
Results: Flowers

Amnis ImageStream[x]









circular interference pattern from center sphere with deformed rings and fringes due to surrounding spheres

misleading, looks like sphere but spots not on same height, maybe due to orientation of flower

Conclusion & Personal Statement

We have successfully shown that low-cost CMOS cameras possess sufficient sensitivity for imaging and angle-resolved scattering of particles in flow. Sideward images allow for the differentiation of single or multiple spherical objects and anisotropic particles. Nevertheless, additional spots – potentially from self-interference on the particle surface - hinder the analysis. The observation of angle-resolved forward scatter yields additional information about the particle orientation and clustering. Although scatter patterns of anisotropic particles are difficult to model with the discrete dipole approximation, our simulations further refine the accuracy of counting particles.

In our future work, we will expand the setup to image the full angle-resolved FSC of the microscope; fine-tune our simulations to cover flower particles, and apply our methods to organic cells to improve counting accuracy.

Since I have a background in diode laser physics, I hardly knew anything about flow cytometry when starting my PhD studies in July 2020 in the department of biomedical optics. I faced the challenge of learning the many constituents of blood, how to work in a S2 safety area or handle pipettes. I am thankful for my colleagues in helping me quickly adapt. Luckily, my ‘laser skills’ prove beneficial in building a new kind of laser flow cytometer!

[1] Discrete Dipole Approximation: https://github.com/adda-team/adda | medium: n(H_2O)=1,34; particles: n(SiO2-Hybrid)=1,41

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