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Multifocus fluorescence correlation spectroscopy

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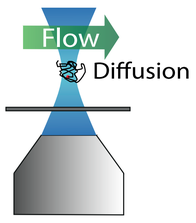
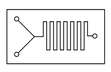
Introduction

Motivation

Flow and diffusion measurement
Small volume (fl)
Low concentration
fluorescence solution (nM and pM)



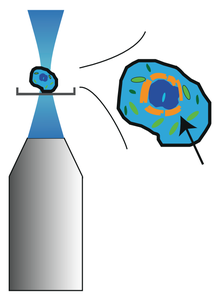
Lab on a chip



Mixing

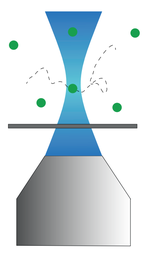
Laminar flow

Molecular motions in cells

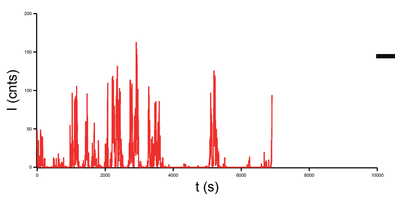


FCS (Fluorescence correlation spectroscopy)

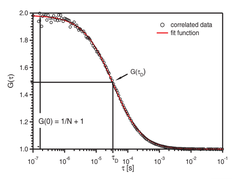
Motion



Fluctuation



Correlation curve



Fit function $G(\tau) = \frac{1}{N}\frac{1}{1+\frac{\tau}{\tau_d}}\sqrt{\frac{\tau}{\tau_d}S^2+1}+1$

Parameters **τ_d** - diffusion time
N - average number of molecules in focus

Problem

- standard one-focus-FCS measurements lack the determination of absolute diffusion coefficients and direction of flow, and cannot diferentiate between flow and diffusion, if

$t_f \sim t_d$

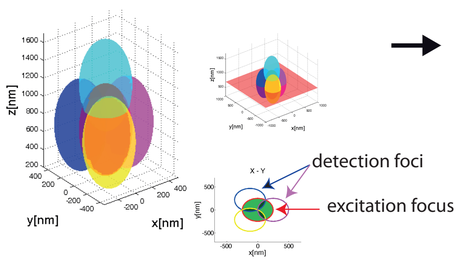
↓

- extension to multifocus-focus-FCS enables **exact** measurement of intracellular transport in terms of **diffusion-coefficient and directed motion**

- **no calibration** due to shifted detection-foci (internal ruler)

Ideas

Multifocus FCS scheme (**one excitation** focus - **four detection** foci)



Global fit of
4 auto- and 12 cross-correlation curves

Parameters

D, conc

+

V_0 - absolute velocity
σ - direction of the flow in xy plane
θ_f - direction of the flow in z
(sphreric angles)

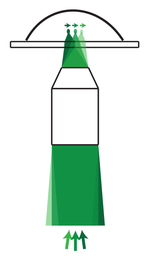
Materials and methods

Experimental realization

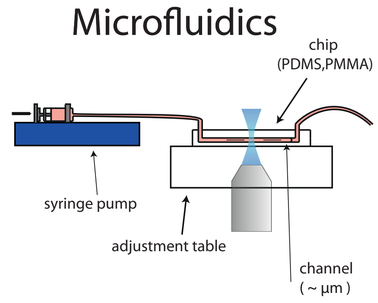
Home build laser scanning confocal microscope

- Las - CW laser (488 nm & 594 nm)
- Fib - single mode optical fiber
- Scan - galvanometric scanner
- Obj - water-immersion objective lens
- Fil - band-pass filter
- Ach - achromat lens
- Bcx - biconvex lens
- λ/2 & λ/4 -half & quarter wave plate
- Dic - dicroic mirror
- Tel - telecentric lenses
- Sam - sample
- BS - 50/50 beamsplitter
- Pin - pinhole
- Det - detector (avalanche photodiode)

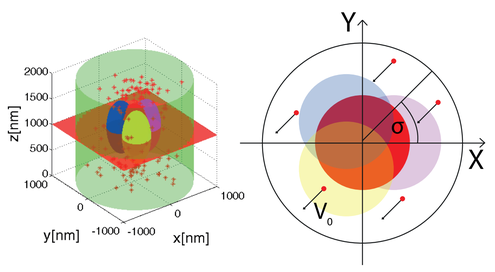
Artificial flow



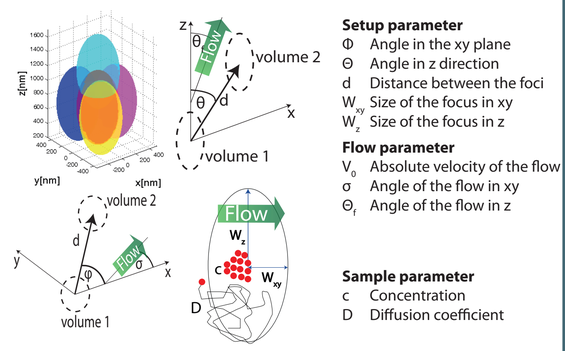
Microfluidics



Simulation



Parameters



Setup parameter
Φ Angle in the xy plane
Θ Angle in z direction
d Distance between the foci
W_{xy} Size of the focus in xy
W_z Size of the focus in z

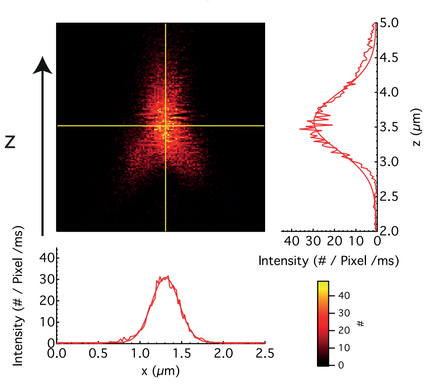
Flow parameter
V_0 Absolute velocity of the flow
σ Angle of the flow in xy
Θ_f Angle of the flow in z

Sample parameter
c Concentration
D Diffusion coefficient

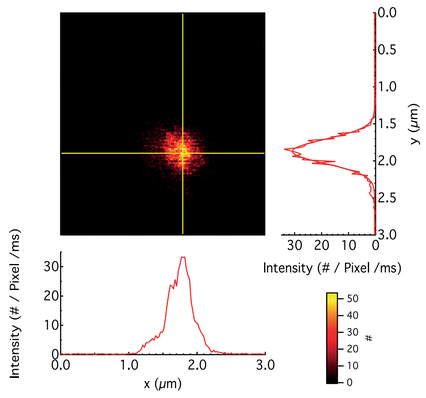
Results

Image of one focus with fluorescent bead

X-Z plane

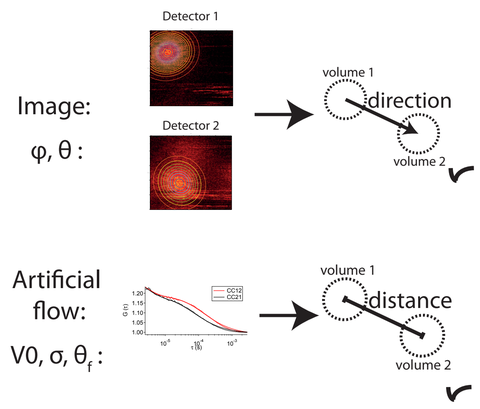


X-Y plane



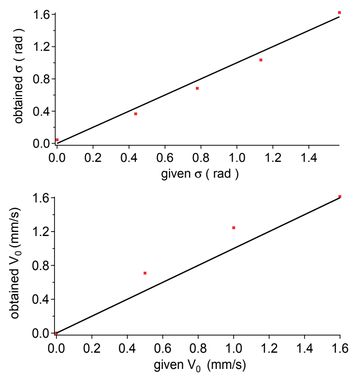
Diffraction limited focus ✓

Calibration



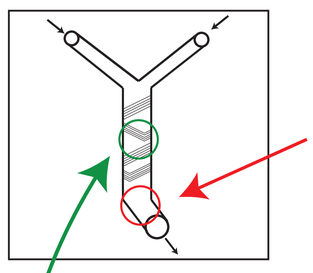
Evaluation

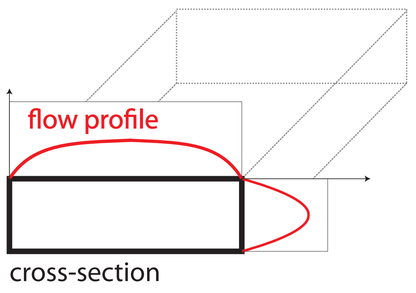
Artificial flow measurements

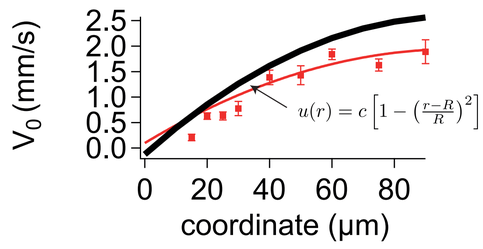


experiment = preset values ✓

Laminar flow

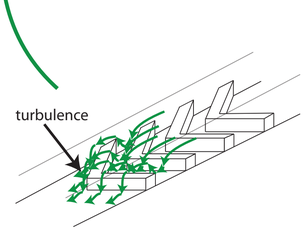


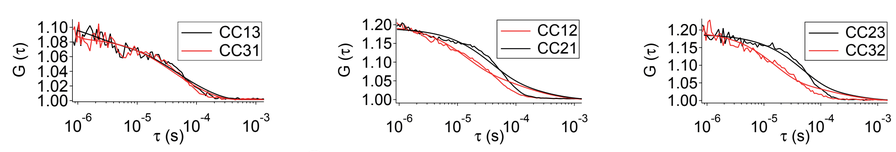




experiment = theory ✓

Turbulent flow





model and experiment doesn't match → ? → possible evidence of turbulent flow

Conclusion & outlook

Dittrich and Schwille[1] as well as Dertinger et al.[2] presented FCS variants with shifted foci for one dimensional flow measurements and for the determination of exact diffusion coefficients.
Following these approaches, we propose four-focus-FCS for the determination of 3D flows.

First experiments on standard fluorophores demonstrate the feasibility of our method.
First studies of flow fields in micro capillary devices have been performed.
In the future, we will assess directed motion in living cells with our experimental scheme.

[1] Dittrich P. S. et al., Analytical chemistry 2002, Vol 74,No 17, 4472-4479
[2] Dertinger T. et al, ChemPhysChem 2007, Vol 8, 433-443

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