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Multifocus fluorescence correlation spectroscopy

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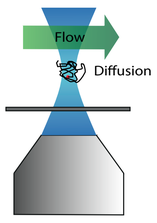
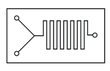
Introduction

Motivation

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



Lab on a chip

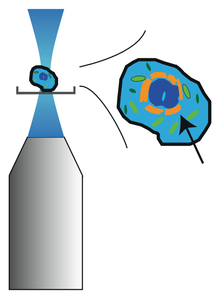


Mixing

Laminar flow

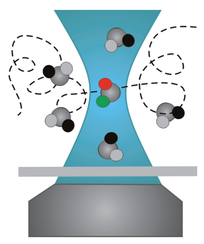
Pulse flow

Molecular motions in cells

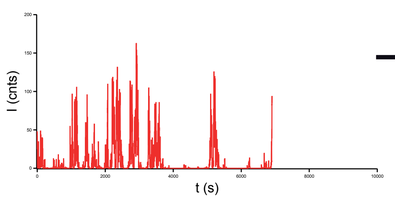


FCS (Fluorescence correlation spectroscopy)

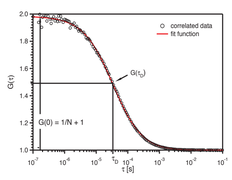
Motion



Fluctuation



Correlation curve



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

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

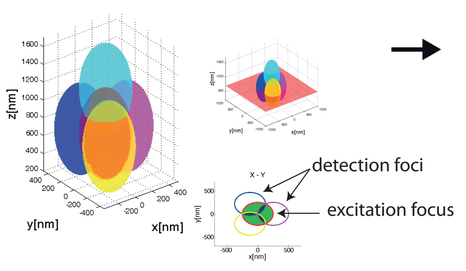
$t_f \sim t_d$

- 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

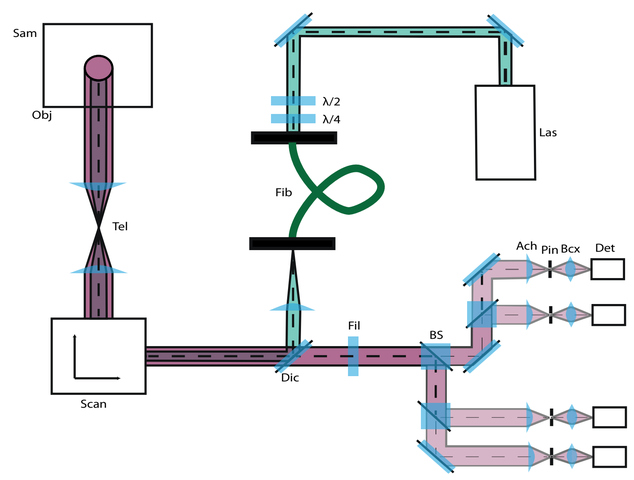
$\tau_{d'}$ N

+

$t_{f,xy}$ - flow time in xy-plane
$t_{f,z}$ - flow time in z direction
σ - direction of the flow in xy plane

Materials and methods

Experimental realization

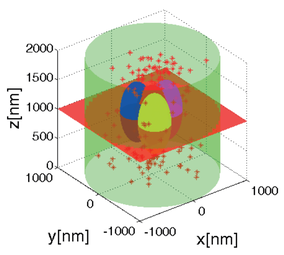


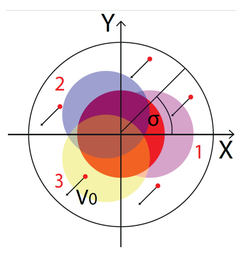
Home build laser scanning confocal microscope

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

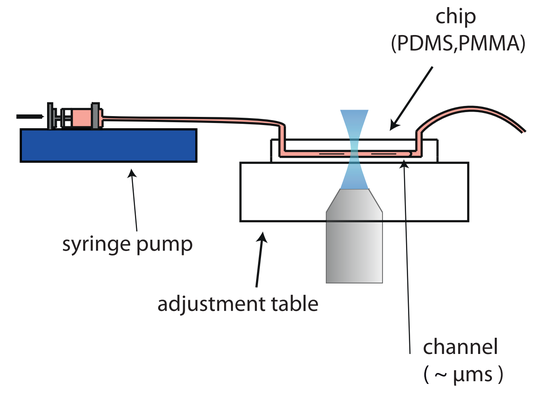
Simulation

3 fFCS simulation





Microfluidics

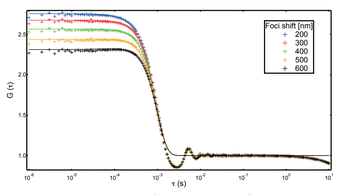


Results

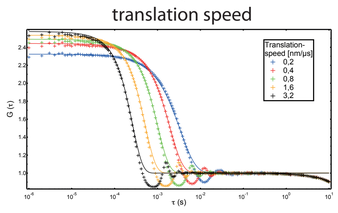
Simulations

theoretically expected correlation functions

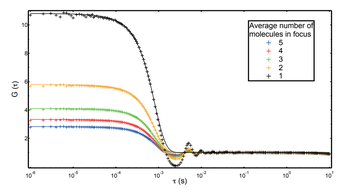
foci shift



translation speed



average number of molecules in focus



translation orientation

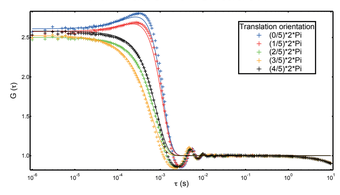
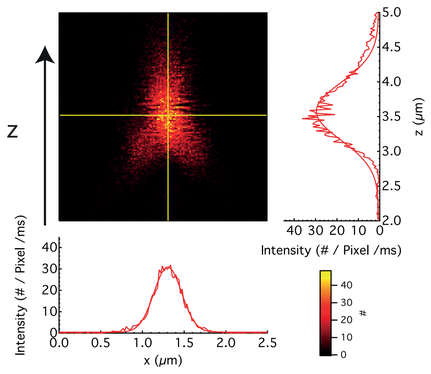
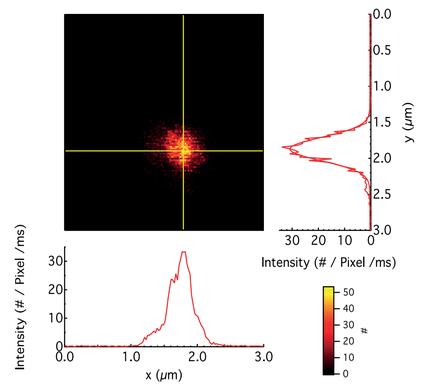


Image of one focus with fluorescent bead

X-Z plane



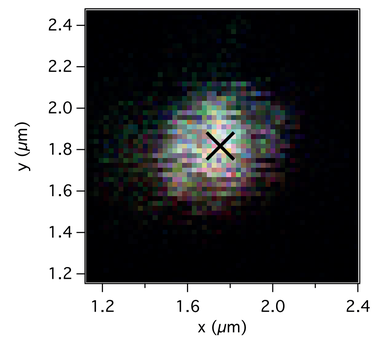
X-Y plane



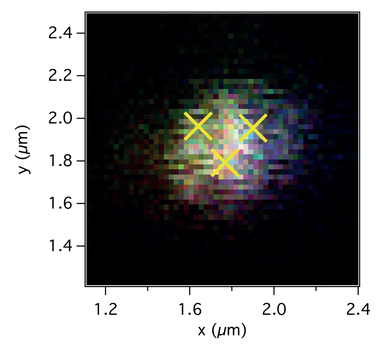
Diffraction limited focus ✔

Image of three foci with fluorescent bead in xy plane

unshifted



shifted

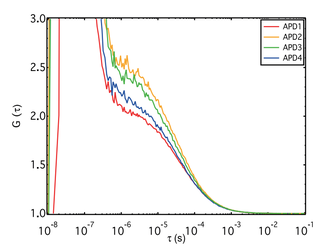


Three distinct foci ✔

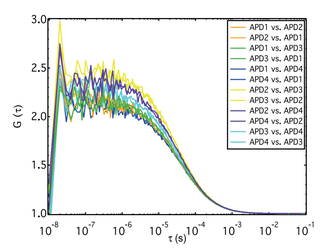
FCS measurements

Alexa Fluor® 488, 1nM solution
laser power 30 µW, diffusion only

All AC curves



All CC curves



Correlation as expected ✔

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 and evaluate this method by simulations.
First experiments on standard fluorophores demonstrate the feasibility of our method.
First studies of flow fields in micro capillary devices are beeing 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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