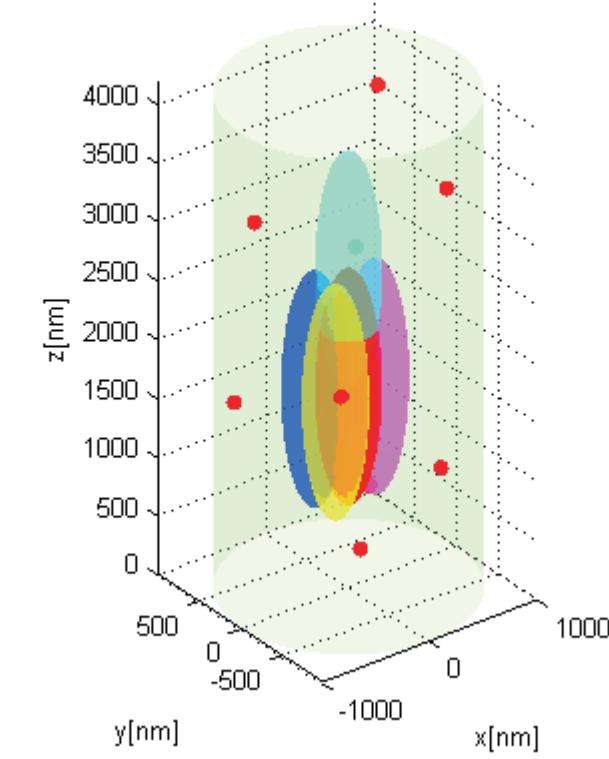


Multifocus-Fluorescence-Correlation-Spectroscopy

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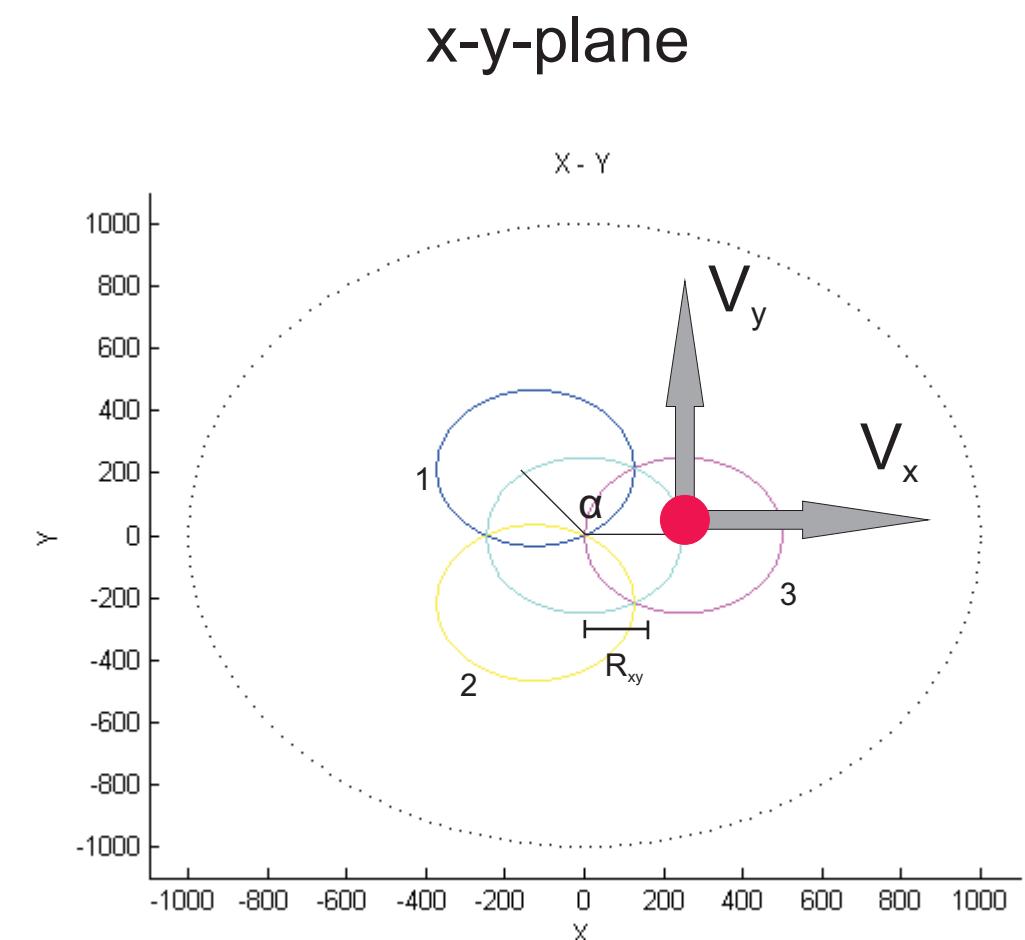
Motivation



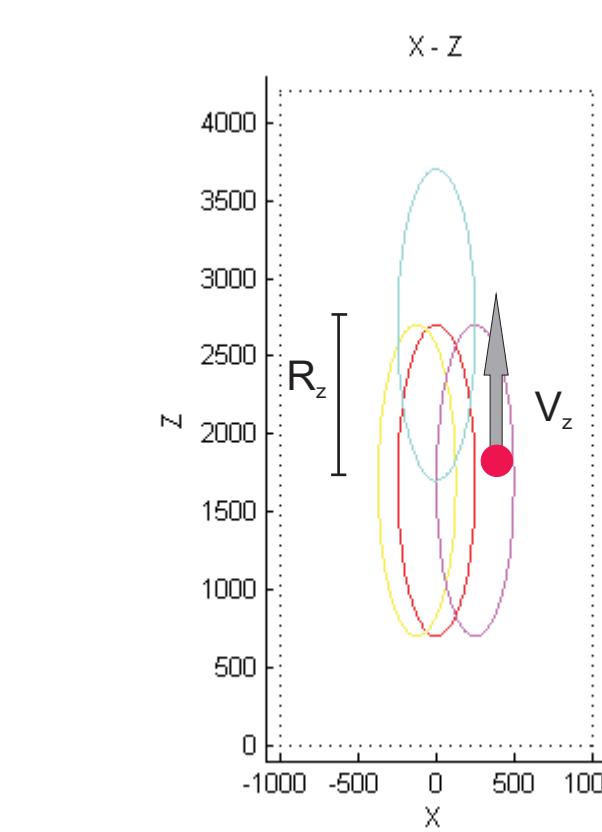
- standard one-focus-FCS measurements lack the determination of absolute diffusion coefficients
- extension to four-foci-FCS enables **exact** measurement of intracellular transport in terms of **diffusion-coefficient and direction**
- **no calibration** due to shifted detection-foci (internal ruler)
- simple experimental implementation

Theory

One excitation focus - four detection foci


 V_x, V_y, \dots velocity of flow; R_{xy}, \dots shift of the 1st, 2nd and 3rd focus in xy-plane from the excitation focus

x-z-plane


 $V_z \dots$ velocity of flow; $R_z \dots$ shift of the 4th detection focus from the excitation focus

Theory of the crosscorrelation function of two foci (m,n)

Crosscorrelation

$$G_{m,n}(\tau) = \langle I_m(t)I_n(t+\tau) \rangle$$

Intensity

$$I_{m/n}(t) = \int C(\vec{r}, t) MDF_{m/n}(\vec{r}, t) d\vec{r}$$

Molecular detection function

$$MDF_{m/n}(\tau) = QI_0 e^{(-\frac{(x-\cos(\alpha_{m/n})R_{xy})^2 + (y-\sin(\alpha_{m/n})R_{xy})^2}{W_{xy}^2} - \frac{(z-R_{z,m/n})^2}{W_z^2})} e^{(-\frac{x^2+y^2}{W_{xy}^2} - \frac{z^2}{W_z^2})}$$

The fitting of the crosscorrelation curves was computed numerically.

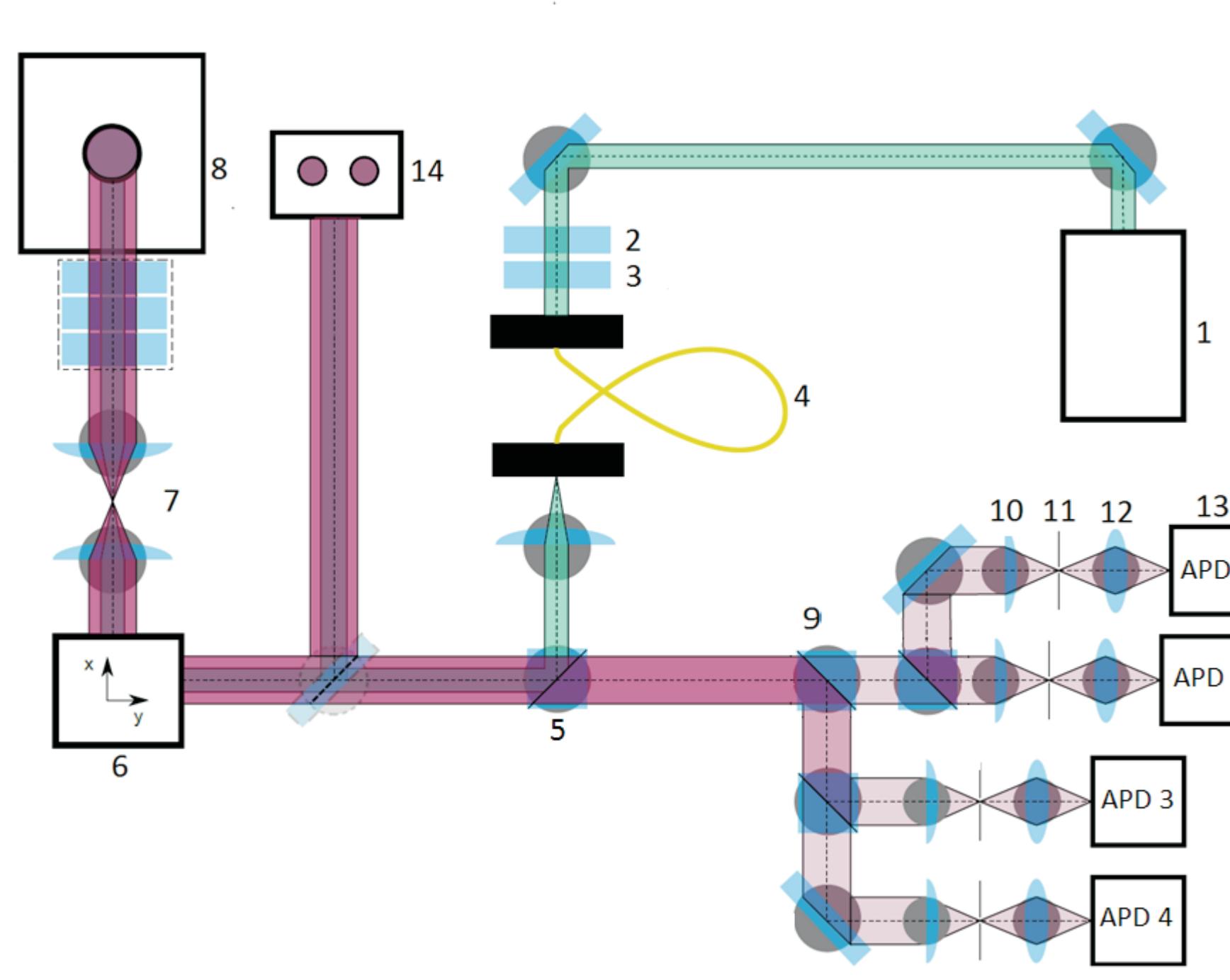
$$\alpha_1 = \frac{2}{3}\pi; \alpha_2 = \frac{4}{3}\pi; \alpha_3 = 0$$

$$G_{1,2} = \frac{e^{(\frac{\tau(3DR_{xy}^2 + \sqrt{3}R_{xy}V_yW_{xy}^2 - \tau W_{xy}^2(V_x^2 + V_y^2))}{4D\tau W_{xy}^2 + W_{xy}^4})}}{(W_{xy}^2 W_z)} + 1$$

$$G_{1,4} = \frac{\sqrt{15}e^{(\frac{1}{20}R_{xy}^2((\frac{2(3+\sqrt{3})}{W_{xy}^2}) - (\frac{5}{4D\tau W_{xy}^2})) + ((\frac{D}{4D\tau W_{xy}^2 + W_z^2}V_z(R_z - \tau V_z)W_z^2)}{4D\tau W_{xy}^2 + W_z^2})}}{4N(4D\tau + W_{xy}^2)\sqrt{4D\tau + W_z^2}} + 1$$

$$G_{auto} = \frac{-\epsilon^2(\frac{V_x^2 + V_y^2}{4D\tau + W_{xy}^2} + \frac{V_z^2}{4D\tau + W_z^2})}{N(4D\tau + W_{xy}^2)\sqrt{4D\tau + W_z^2}} + 1$$

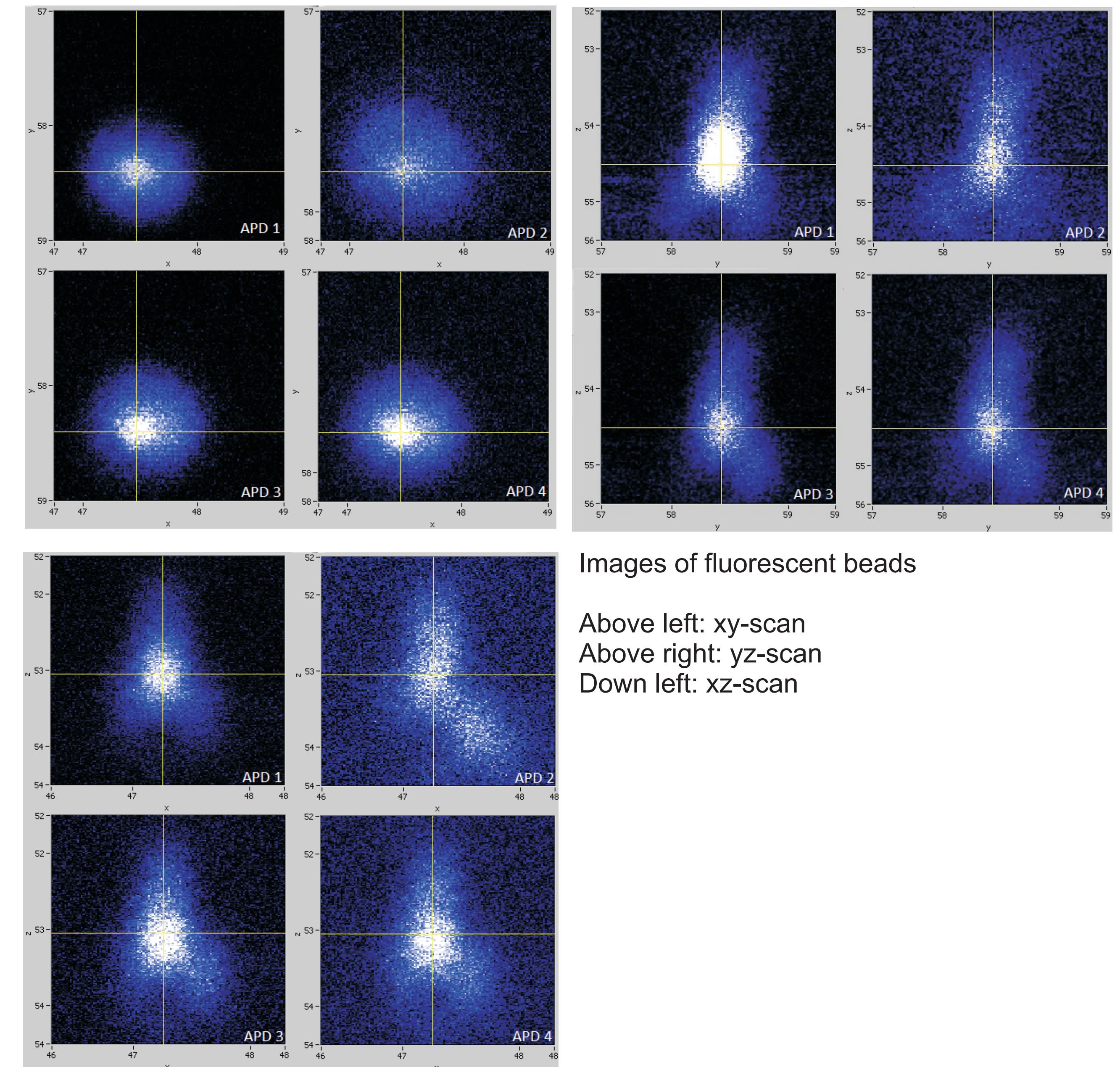
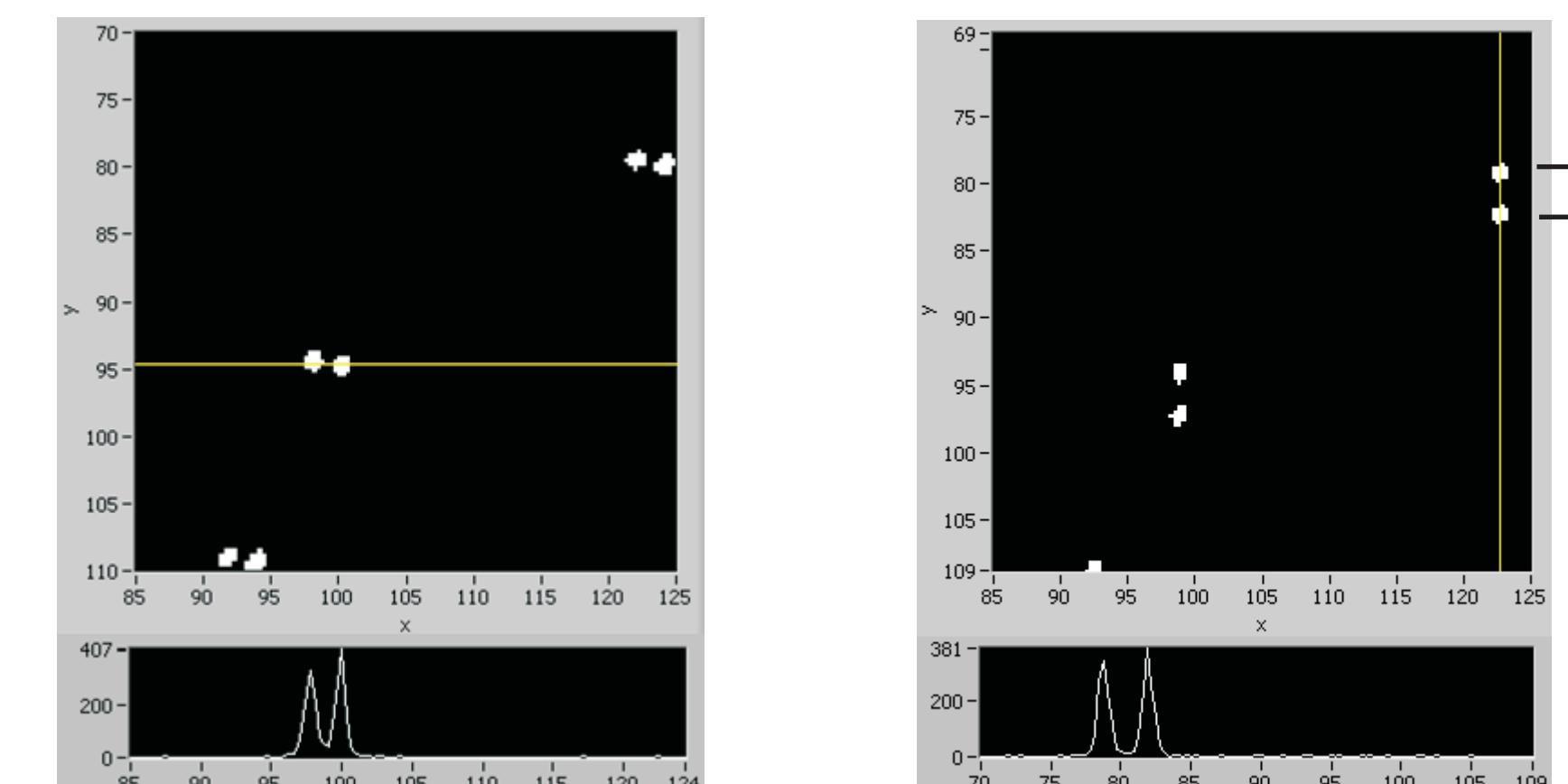
Experimental realization



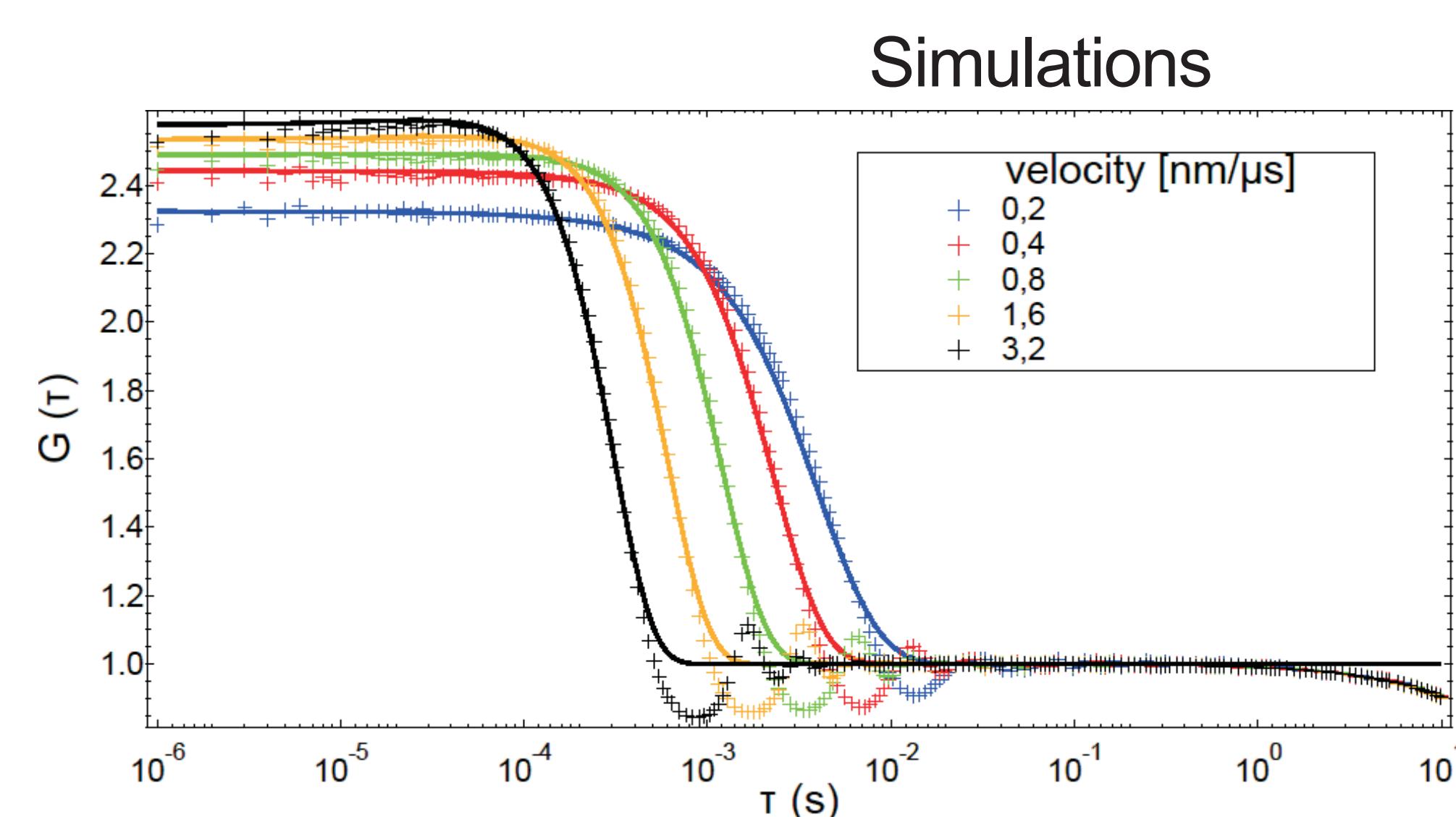
- laser
- N/4-plate
- N/2-plate
- optical fiber
- dichroic mirror
- galvanometer scanner
- telecentric-lens-system
- objective + sample
- 50/50-beam-splitter
- achromatic lens
- pinhole
- biconvex lens
- APD (detector)
- microscope-ocular
- microscope-ocular

Results - First images, simulations

Superposition of 2 xy-scan images of fluorescent beads, after shift of pinhole-detector assembly in x- and y-direction



Images of fluorescent beads

Above left: xy-scan
Above right: yz-scan
Down left: xz-scan

The cross correlation $G(t)$ between 1st focus and 2nd focus are shown for various velocity of flow

Conclusion & Outlook

Dittrich and Schwille¹ 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.

Future applications of our method range from detailed studies of flow fields in micro capillary devices to the study of directed motion in living cells.

References

- [1] P. S. DITTRICH, P. SCHWILLE: Spatial Two-Photon Fluorescence Correlation Spectroscopy for Controlling Molecular Transport in Microfluidic Structures. *Analytical Chemistry*, 74 No. 17:4472-4479, 2002.
- [2] T. DERTINGER, V. PACHECO, I. VON DER HOCHT, R. HARTMANN, I. GREGOR, J. ENDERLEIN : Dual-focus Fluorescence Correlation Spectroscopy: A New Tool for Accurate and Absolute Diffusion Measurements. *ChemPhysChem*, 8:433-443, 2007