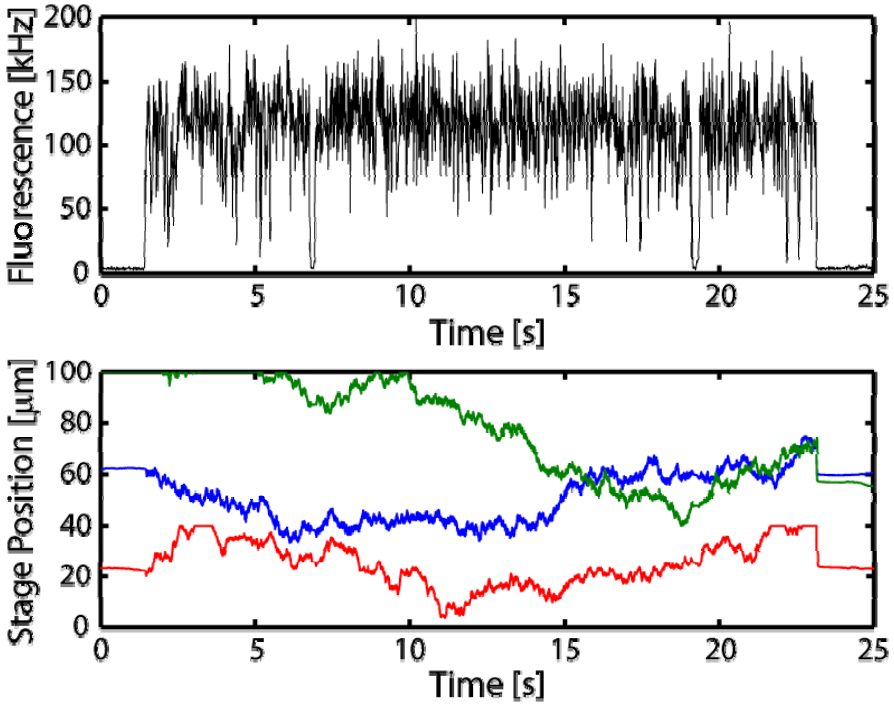
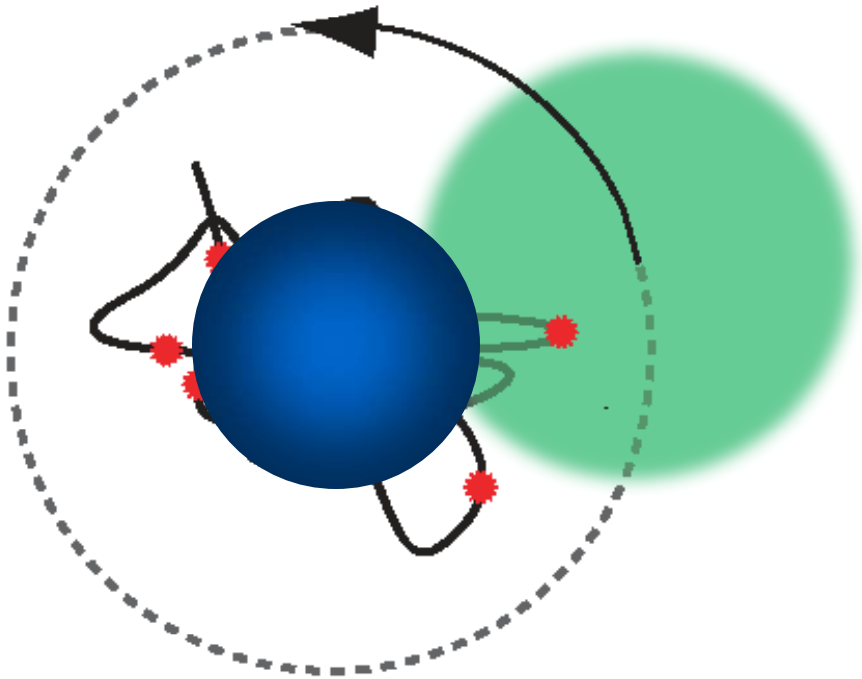
Tracking Fluorescence Correlation Spectroscopy of individual biomolecules

Kevin McHale[1], Andrew Berglund[2], Mike Zhang, Charles Limouse, Chandra Raman[3], Hideo Mabuchi

Edward L. Ginzton Laboratory, Stanford University

[1]Chemical Physics Laboratory, NIDDK, NIH [2]CNST Nanofabrication lab, NIST [3]Physics, Georgia Institute of Technology



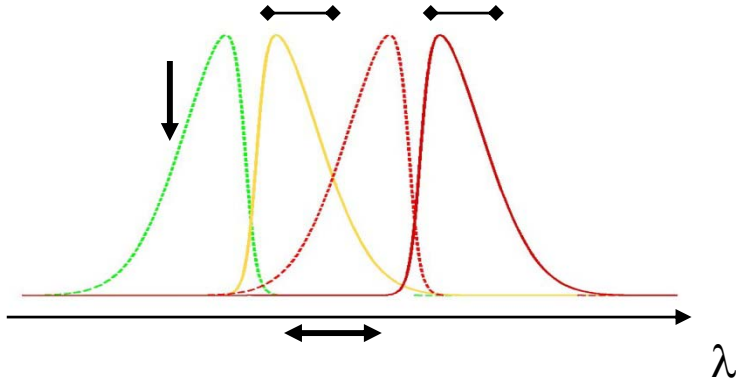


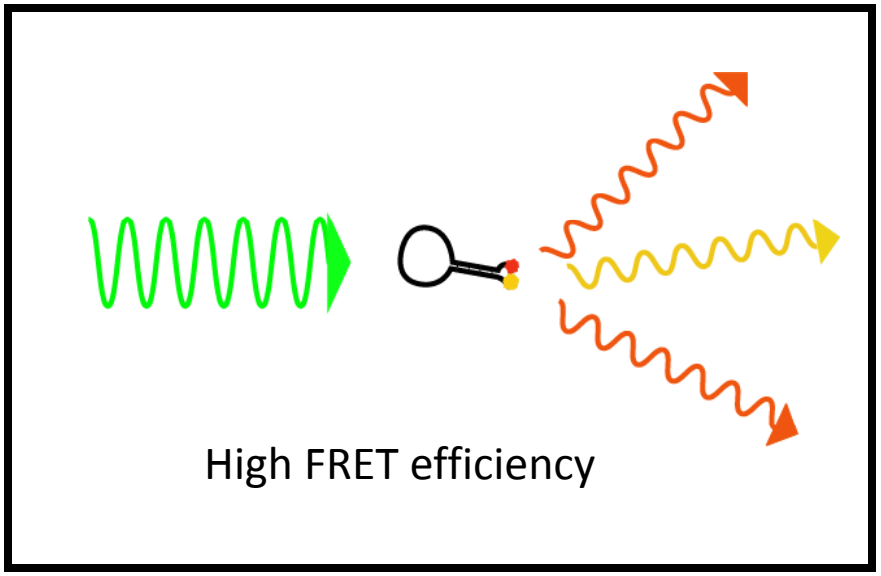
ARO, NDSEG, NSF, NIGMS

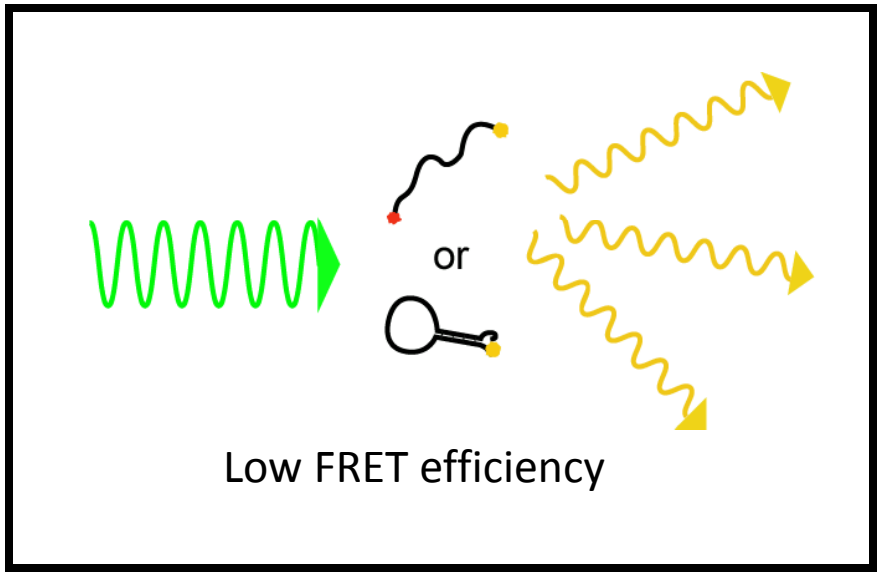
Fluorescence resonance energy transfer (FRET)

- single DNA strands labeled with fluorescent dye molecules

- Position, orientation and spectral overlap determine FRET efficiency

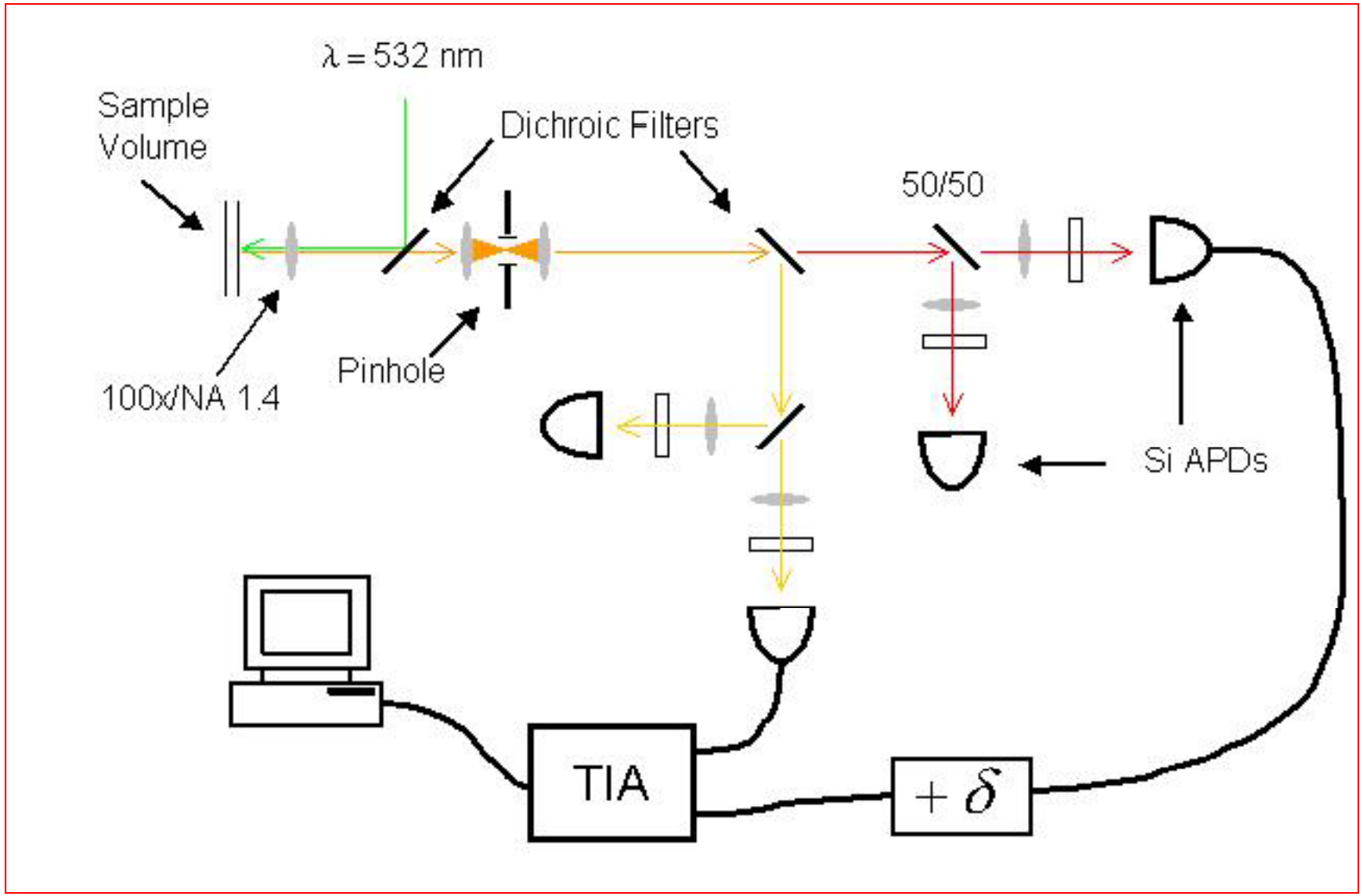




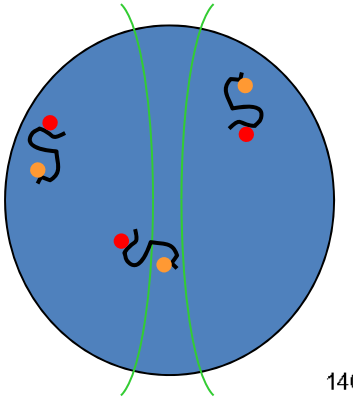


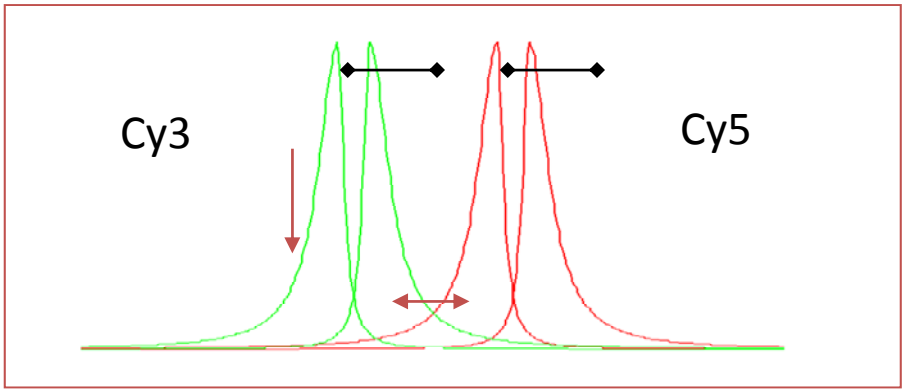
Single-pair FRET measurements on dye-labeled DNA

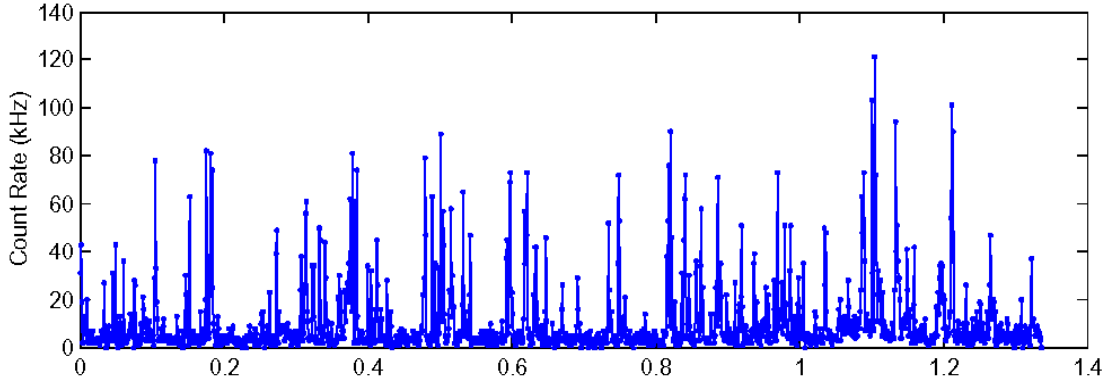
S. Chu *et al*, S. Weiss *et al*

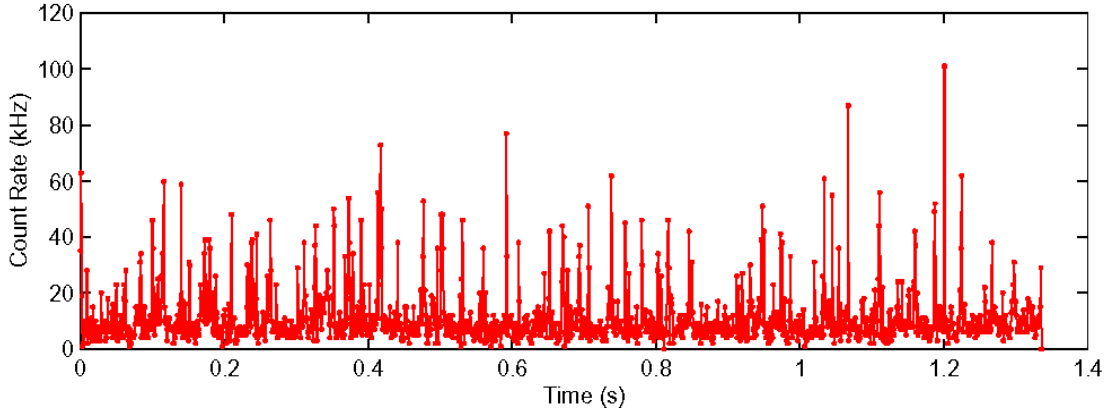


Single-pair FRET measurements on dye-labeled DNA









fluor. ratio

⇩

FRET efficiency

⇩

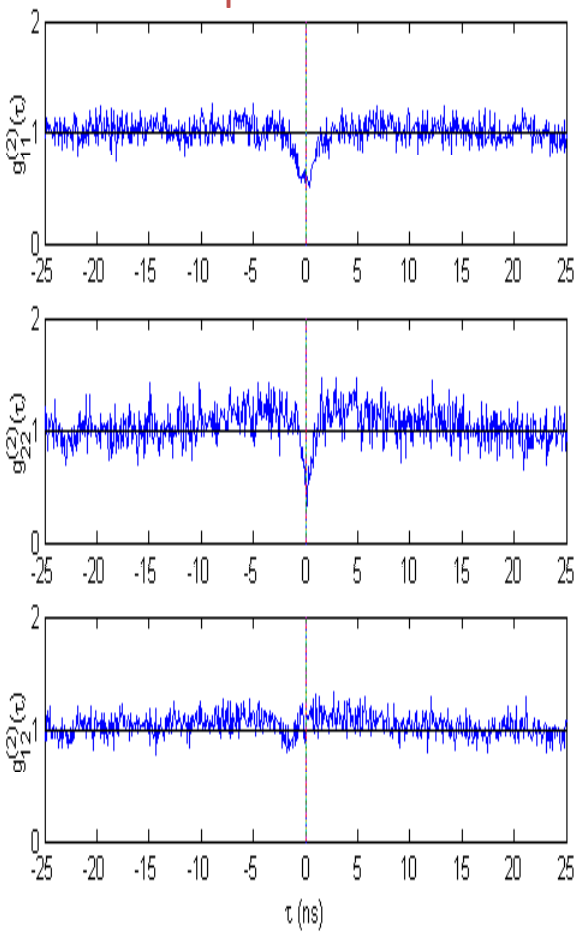
relative position, orientation of dyes

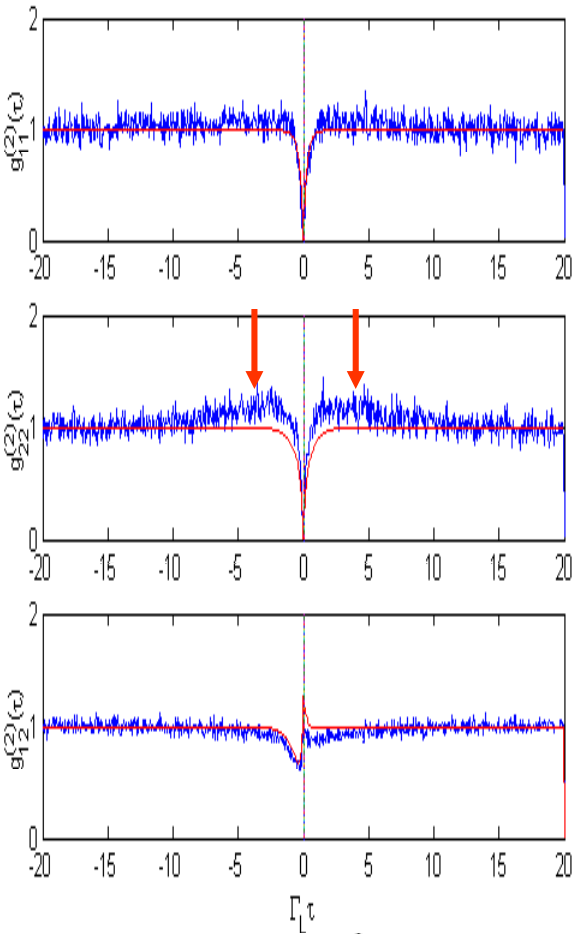
Photon statistics and dynamics of FRET

A. J. Berglund, A. C. Doherty and HM, Phys. Rev. Lett. **89**, 068101 (2002)

Experiment

Simulation

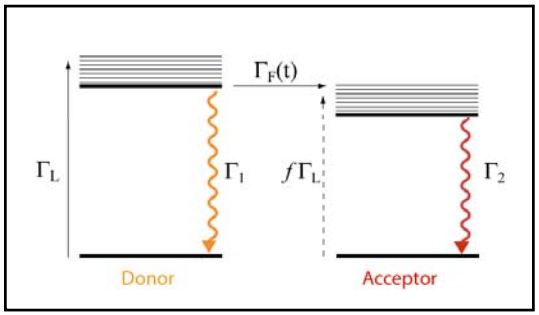


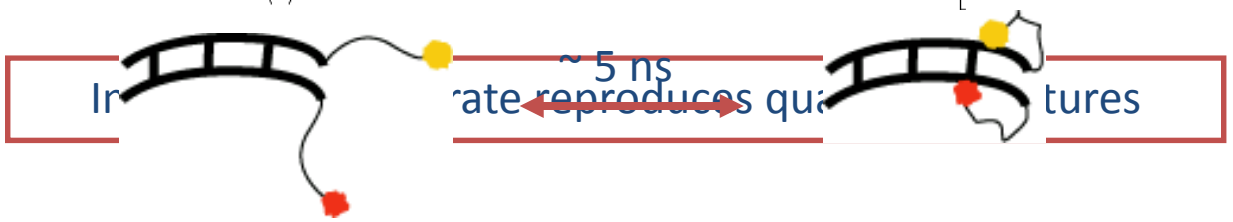


Donor autocorrelation

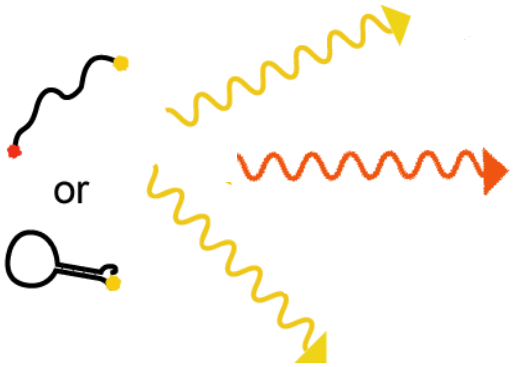
Acceptor autocorrelation

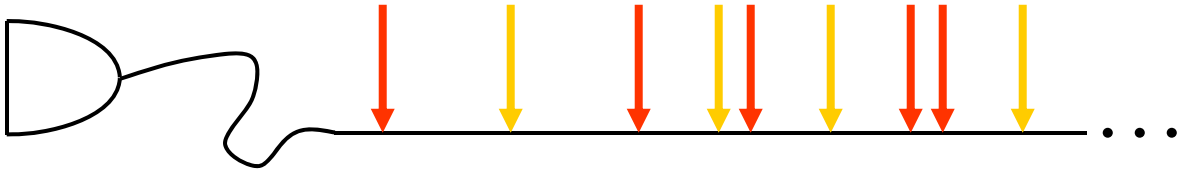
Cross-correlation





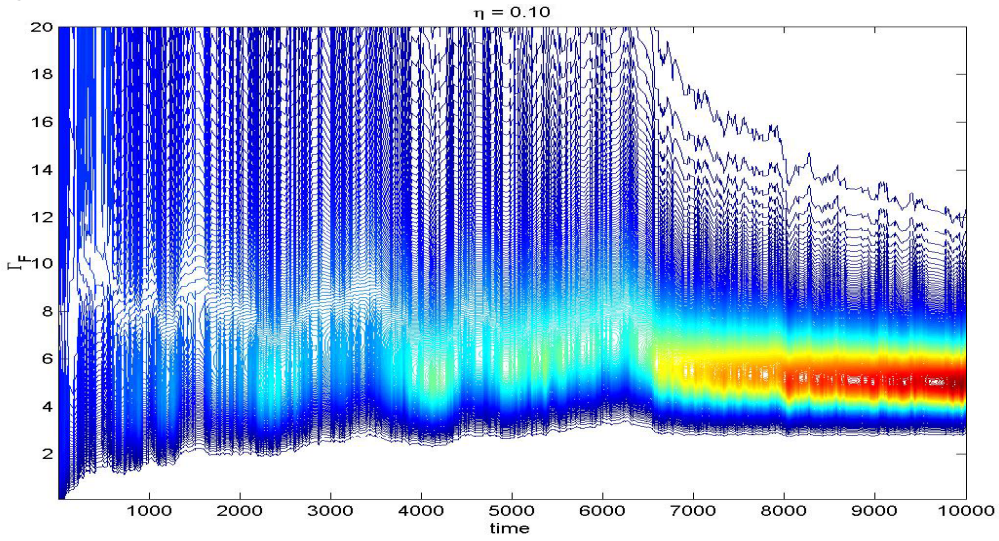
Photon-stream filtering: dynamics vs. shot-noise

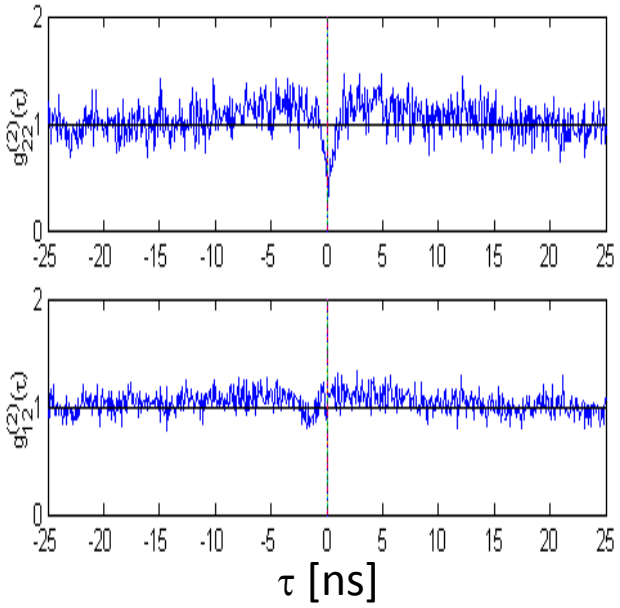


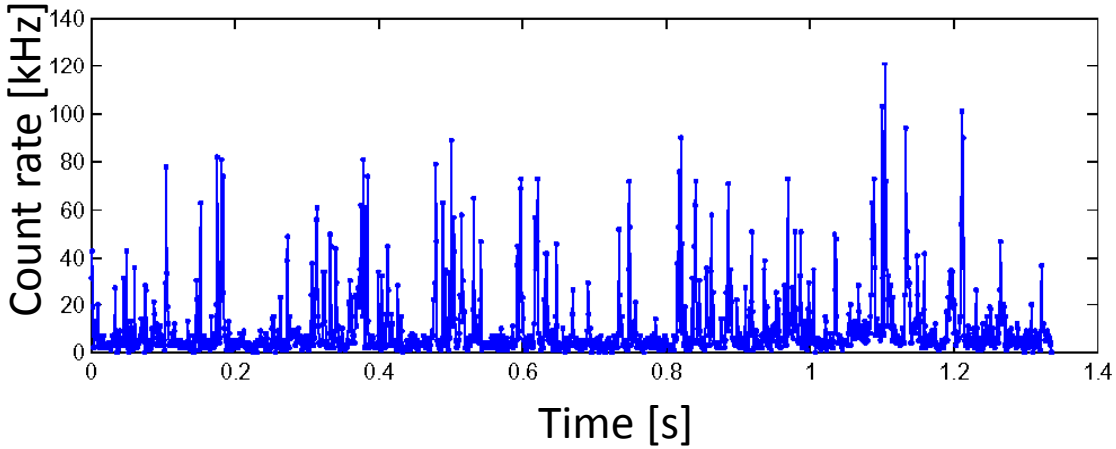


$$p\left(\vec{\theta}\,|\,\Xi[0,t]\right) = q\left(\Xi(t)\,|\,\vec{\theta}\right)\; p\left(\vec{\theta}\,|\,\Xi[0,t)\right)$$

$$d\vec{\theta}_t = \mathcal{A}_t\,\vec{\theta}_t\,dt + \mathcal{K}_t\left[d\Xi_t - C\vec{\theta}_t\,dt\right]$$



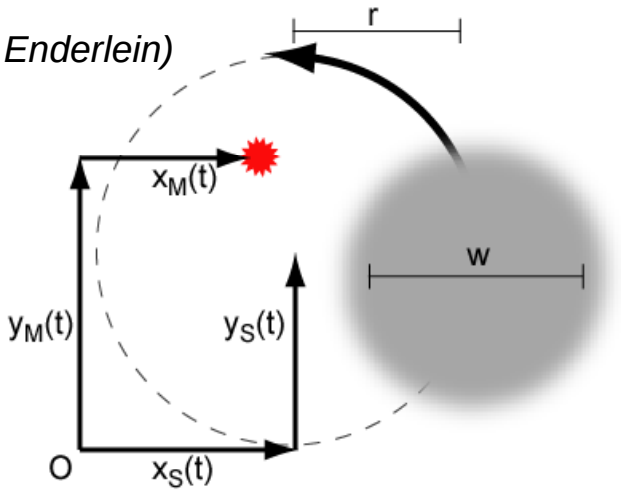


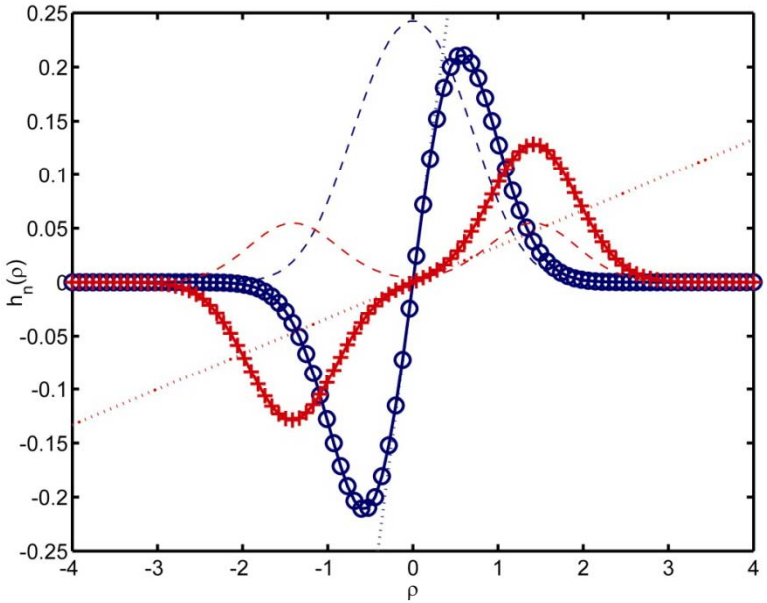


Fluorescence-modulation particle tracking (2D)

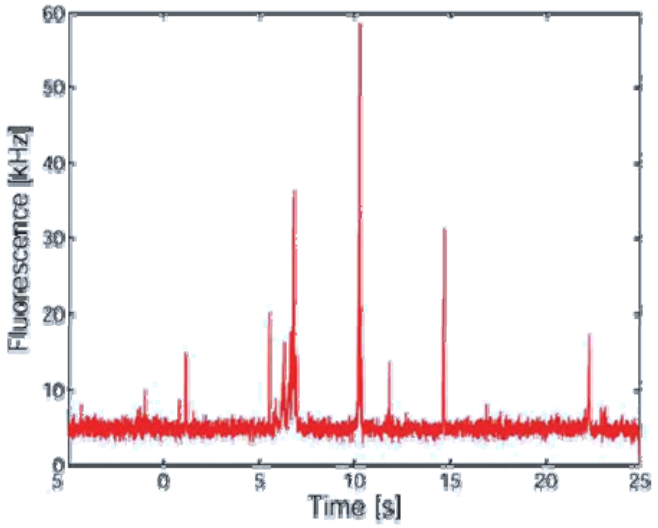
A. J. Berglund and HM, Appl. Phys. B **78**, 653 (2004)

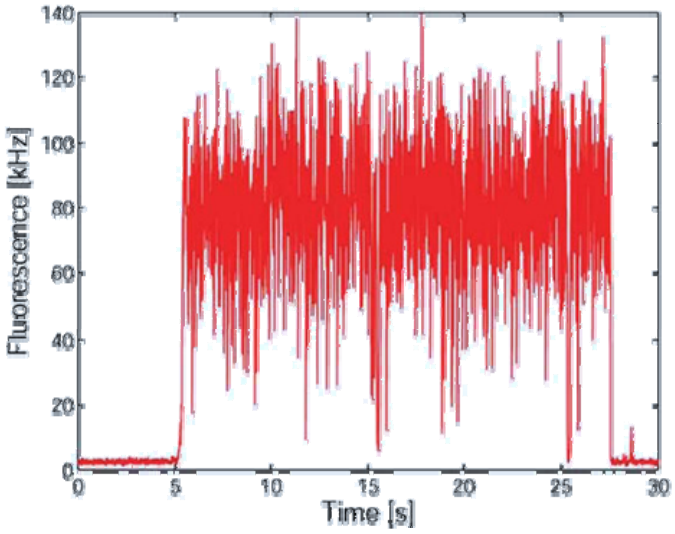
(J. Enderlein)





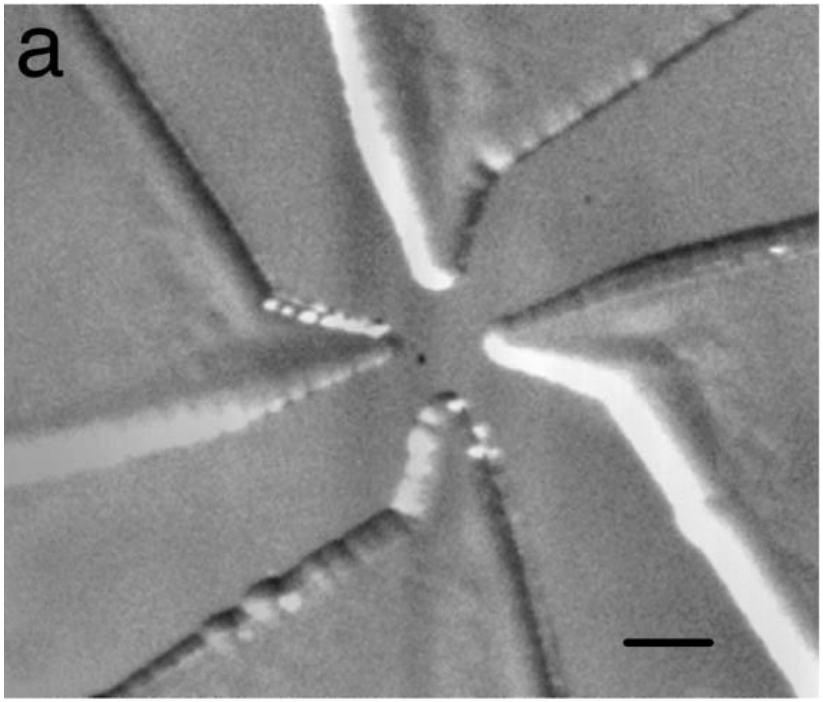
Near axis of rotation the demodulated fluorescence provides a linear error signal

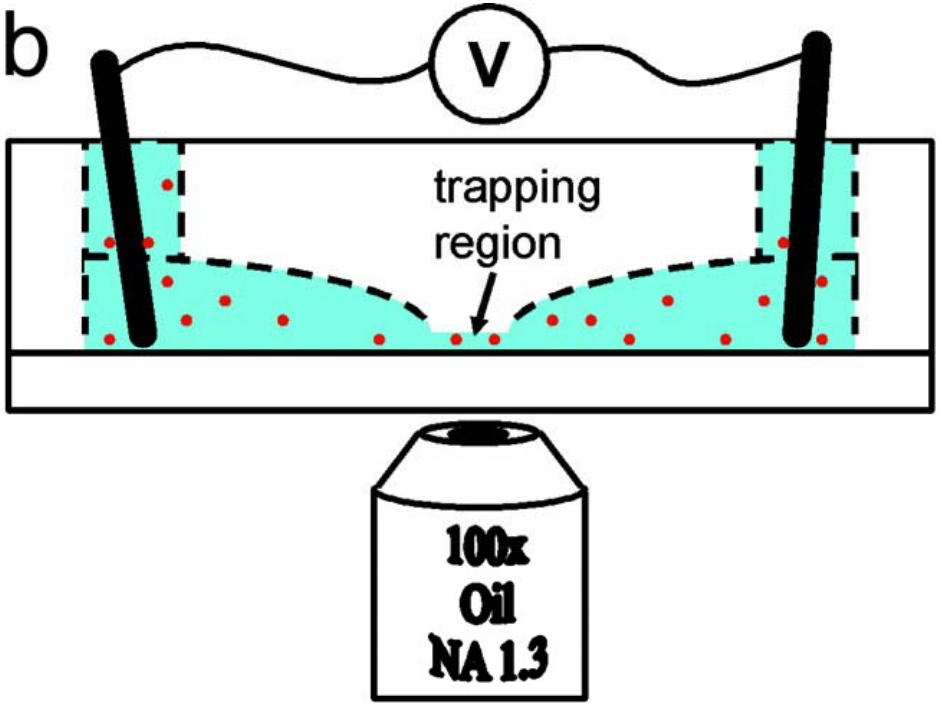




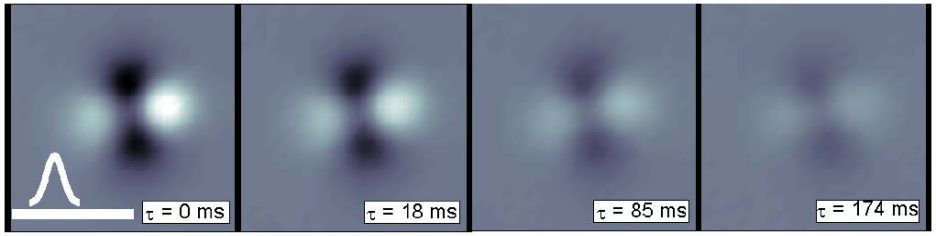
Cohen & Moerner: anti-Brownian electrokinetic trap

A. E. Cohen and W.E. Moerner, Proc. Natl. Acad. Sci. U.S.A. **103**, 4362 (200



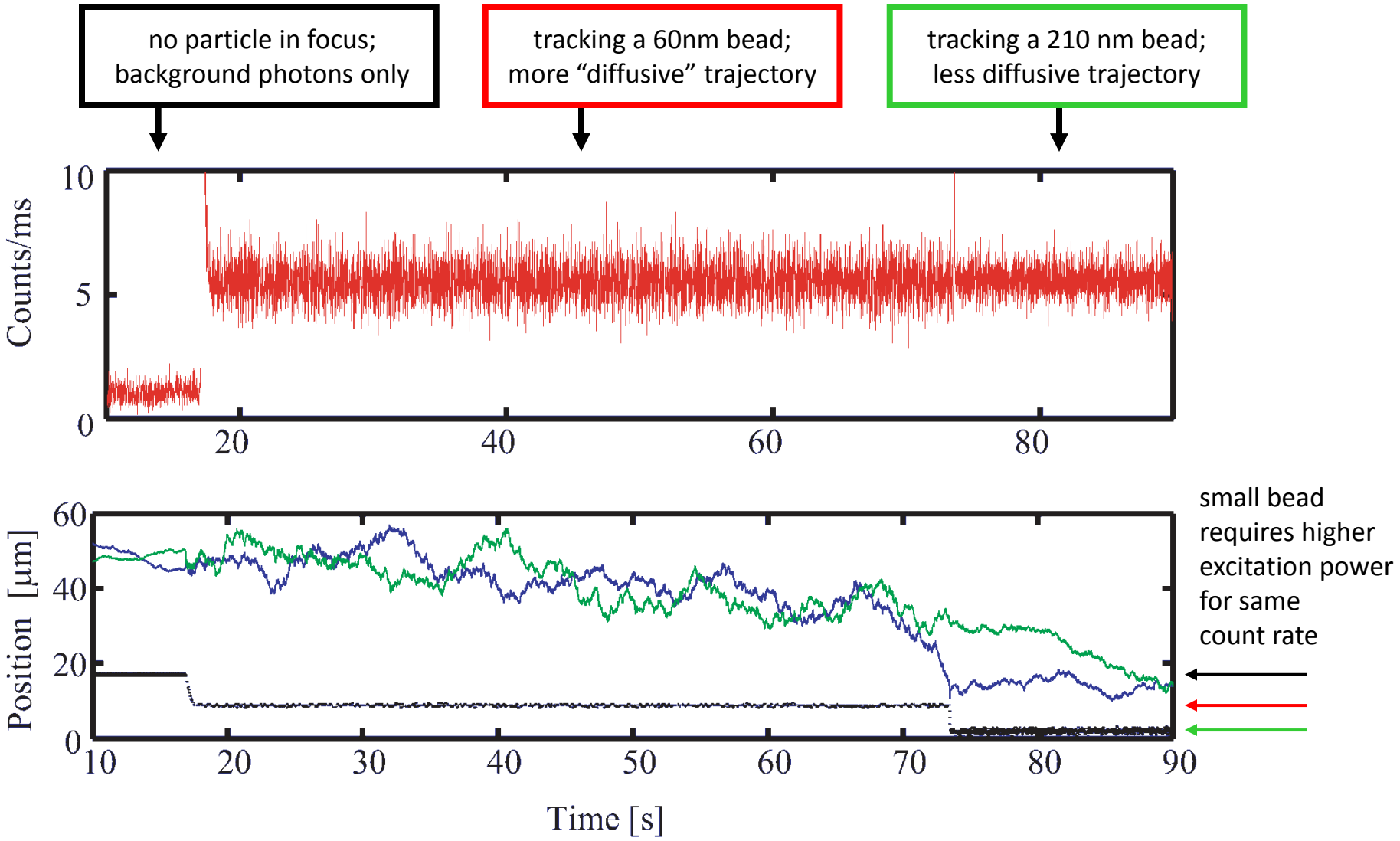


A. E. Cohen and W. E. Moerner, Phys. Rev. Lett. **98**, 116001 (2007)



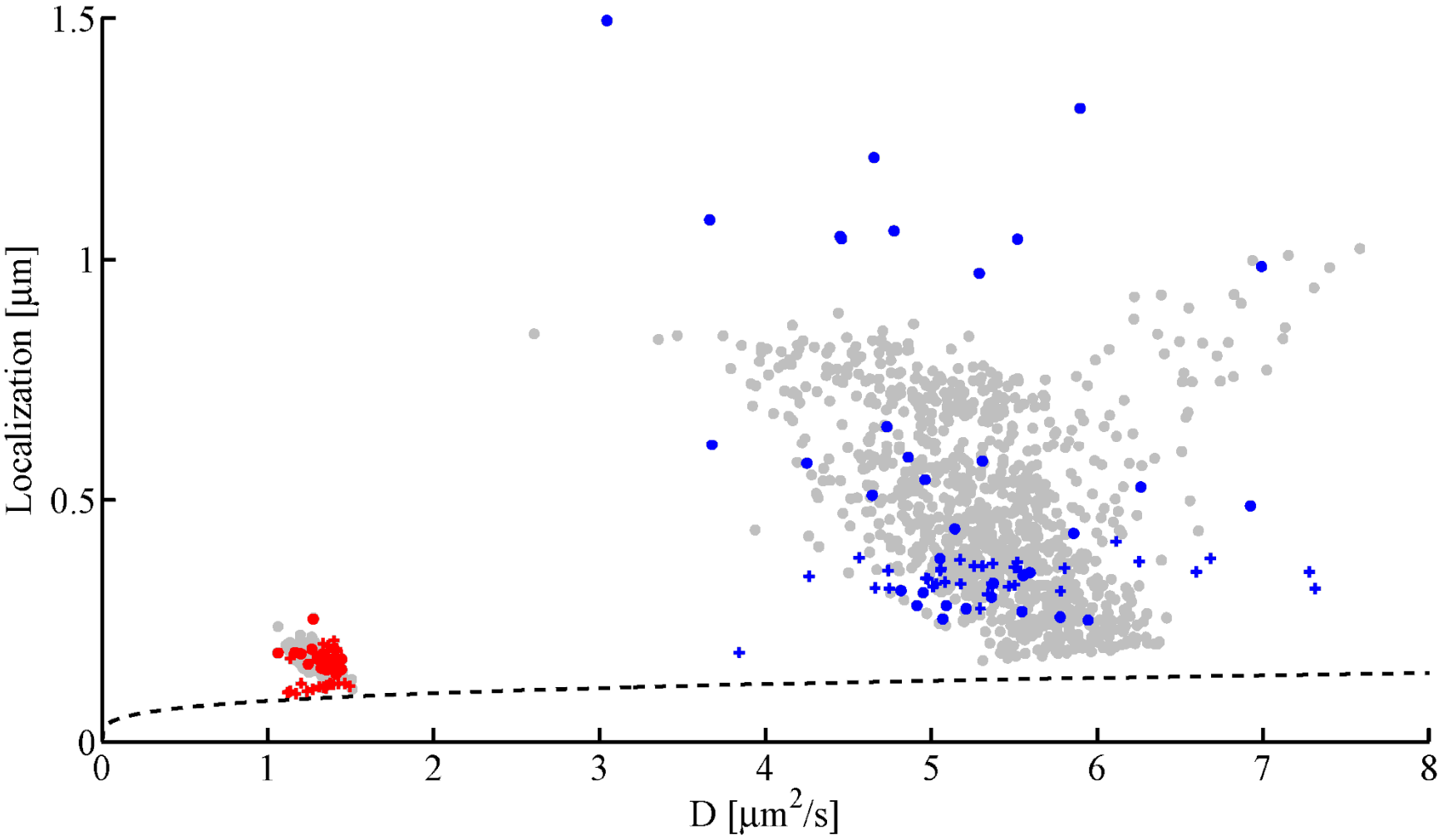
Tracking data channel(s)

A. J. Berglund, K. McHale and HM, Opt. Lett. **32**, 145 (2007)



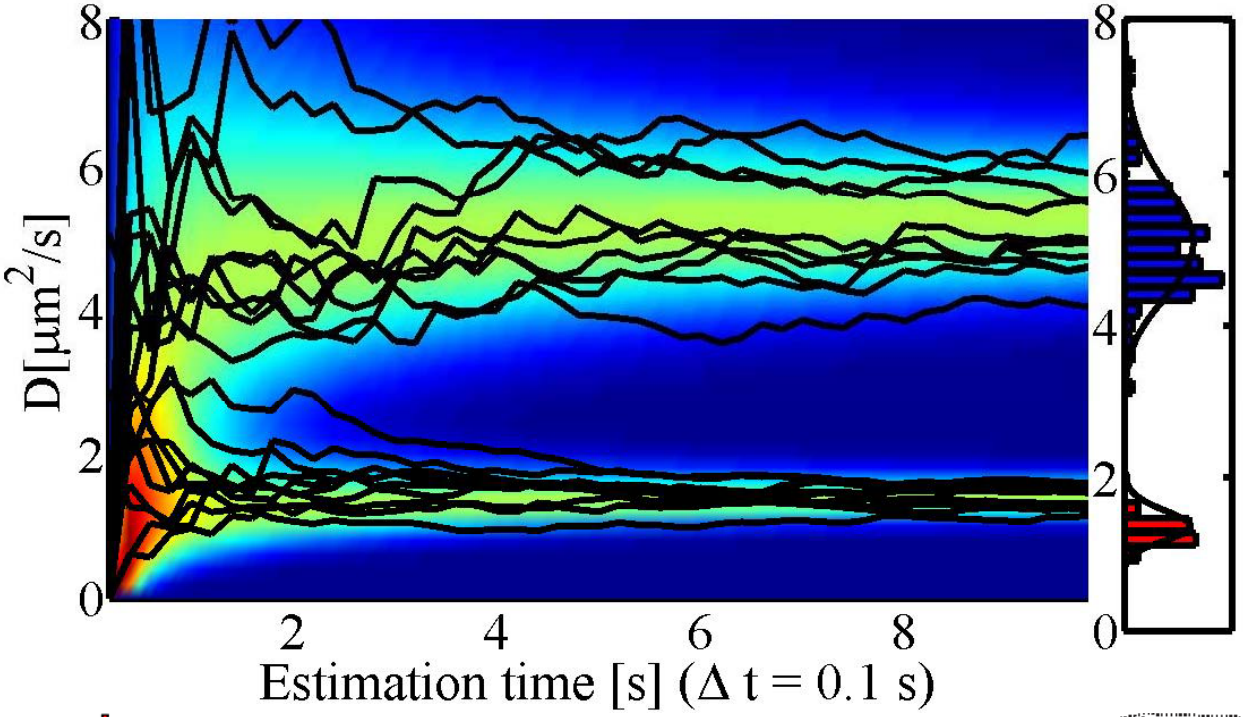
Tracking performance near the shotnoise limit (2D)

A. J. Berglund, K. McHale and HM, Opt. Lett. **32**, 145 (2007)

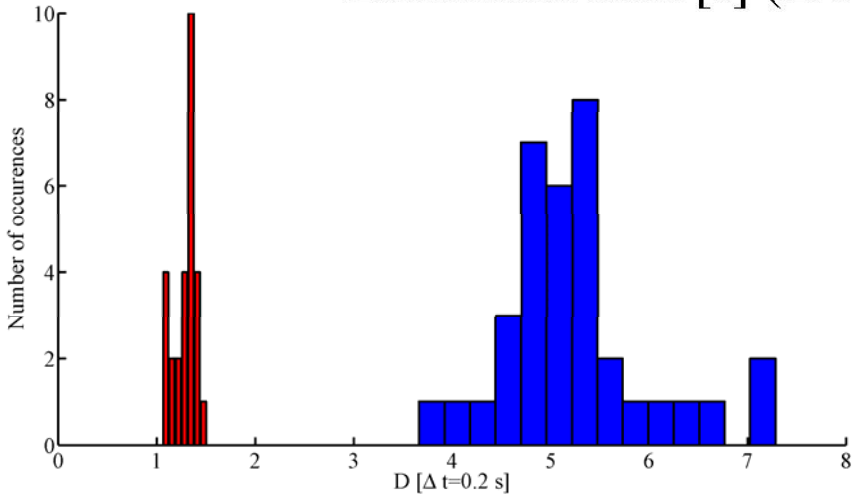


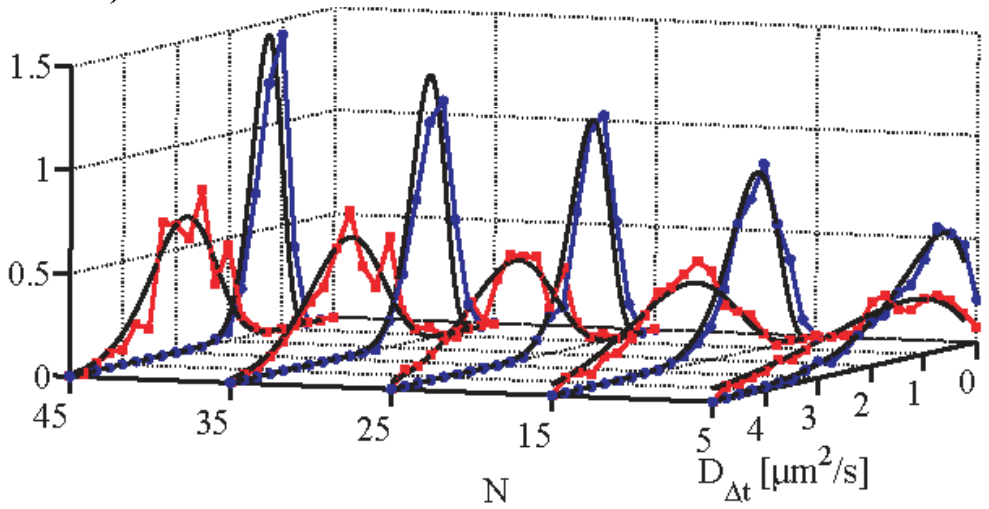
Fast classification of individual nano-particles (beads)

A. J. Berglund, K. McHale and HM, Opt. Express **15**, 7752 (2007)



90% confidence with only ~600 photons!

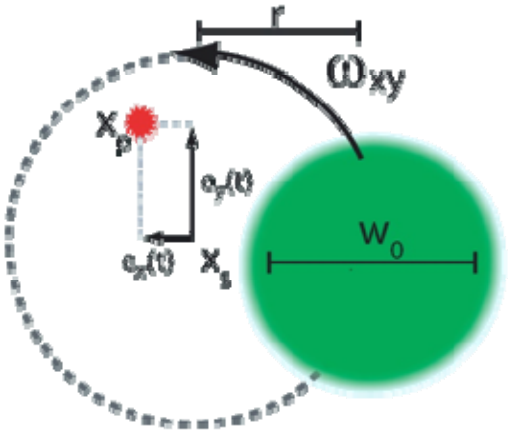




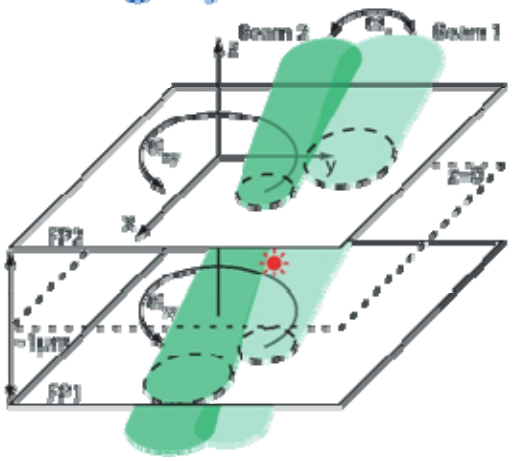
3D Feedback tracking microscopy

Kevin McHale, A. J. Berglund and HM, Nano Lett. **7**, 3535 (2007)

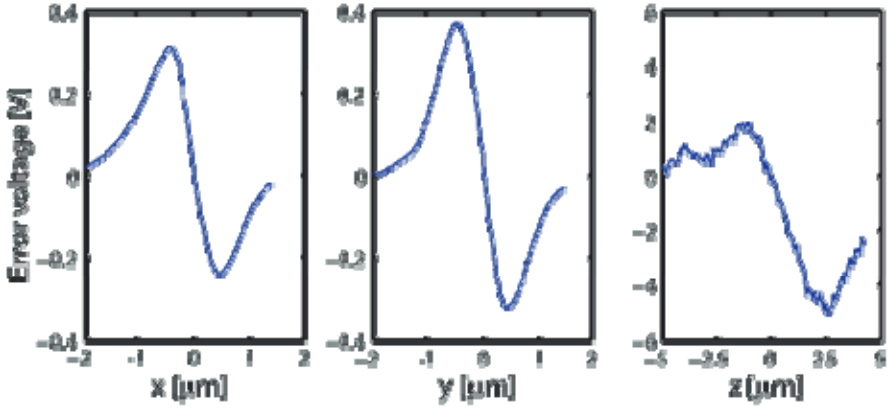
2-D sensing by beam rotation



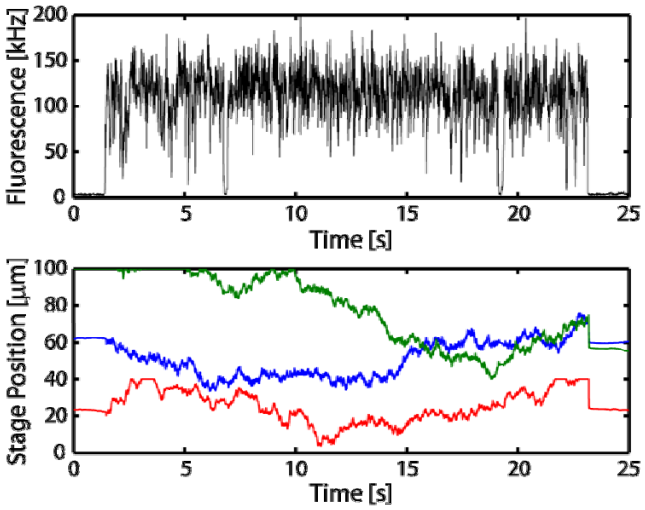
3-D sensing by beam switching



Position error estimates

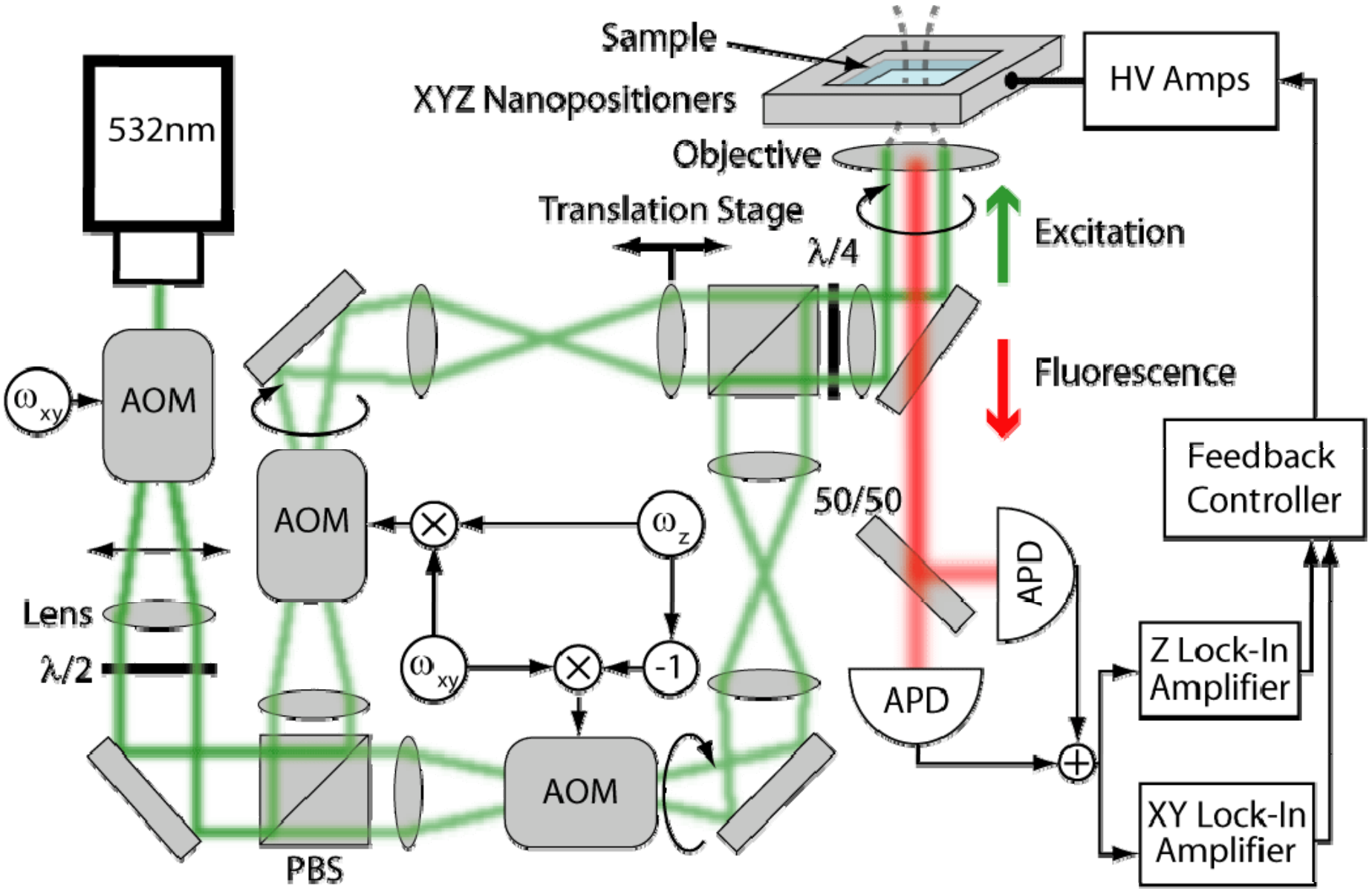


3-D tracking

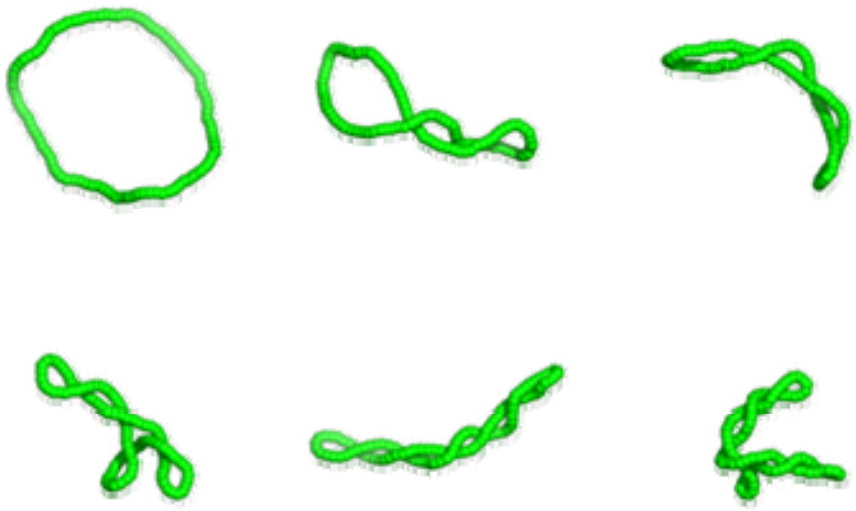


Schematic apparatus

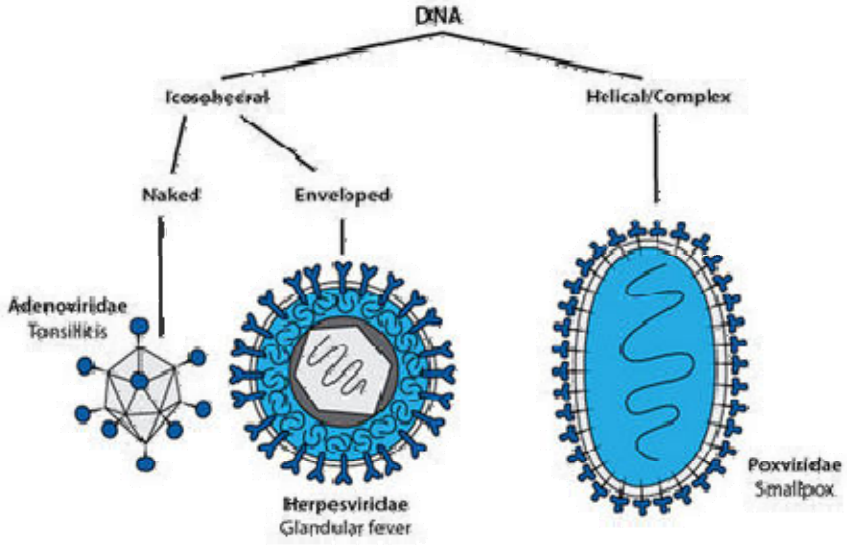
Kevin McHale, A. J. Berglund and HM, Nano Lett. **7**, 3535 (2007)



Free-solution dynamics of large dsDNA molecules



A. J. Spakowitz, Stanford University



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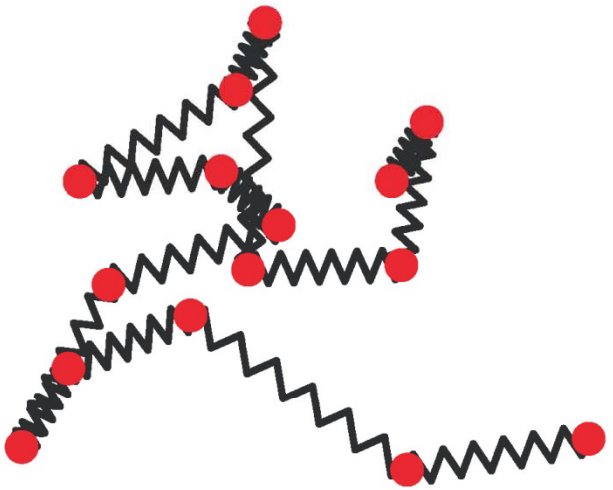
"Simple" (linearized) polymer models

- Rouse: Beads and springs
- Zimm: Hydrodynamic couplings
- Semi-flexible: Bending energy
- None: excluded volume



Question: which (if any) model quantitatively describes DNA?

Experiments on genomic lambda-DNA

K. McHale and HM, submitted

References

- Initial *D* measurements: Smith, Perkins & Chu, *Macromolecules*, 1995
- Intramolecular fluorescence on trapped DNA: Quake, Babcock & Chu, *Nature*, 1997
- Intramolecular FCS of DNA: Lumma, Keller, Vilgis & Rädler, *PRL*, 2003; Shusterman, Alon, Gavrinyov & Krichevsky, *PRL*, 2004; Winkler, Keller & Rädler, *PRE*, 2006; Petrov, Ohrt, Winkler & Schwille, *PRL*, 2006
- DNA in ABEL trap: Cohen & Moerner, *PRL* and *PNAS*, 2007

Lumma *et al.*, Figure 1

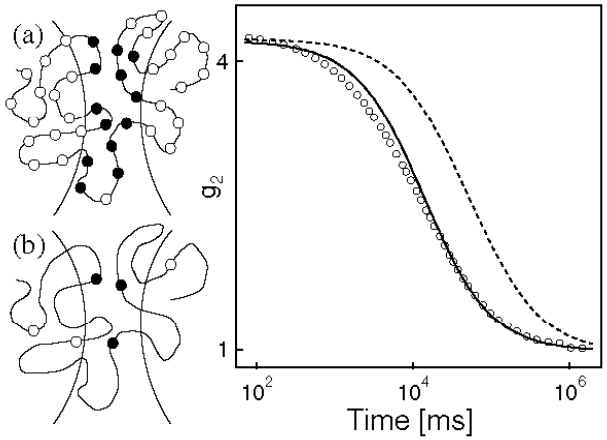
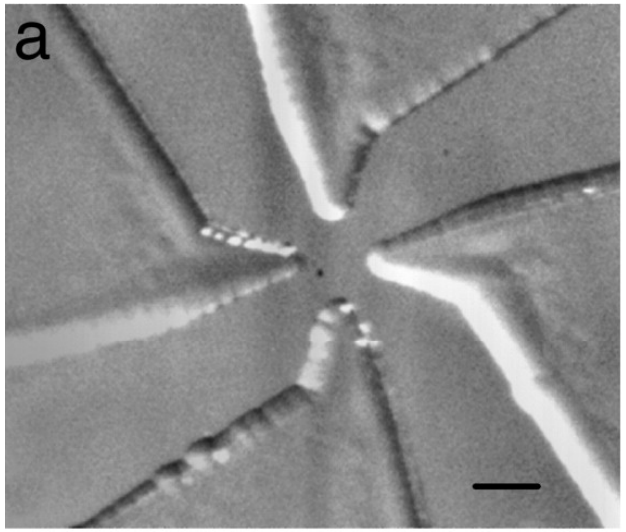


FIG. 1. The measured autocorrelation curve g_2 for dilute λ-phage DNA deviates from the correlation function expected for a stiff finite-size polymer with $R_g = 0.73\ \mu$m (dashed line). The solid line shows the poor agreement with a best fit to the correlation function of a pointlike particle. The drawings illustrate labeled DNA as a chain of linked chromophores for (a) high and (b) low average label densities.

A. E. Cohen and W.E. Moerner
Proc. Natl. Acad. Sci. U.S.A. **103**, 4362 (2006)

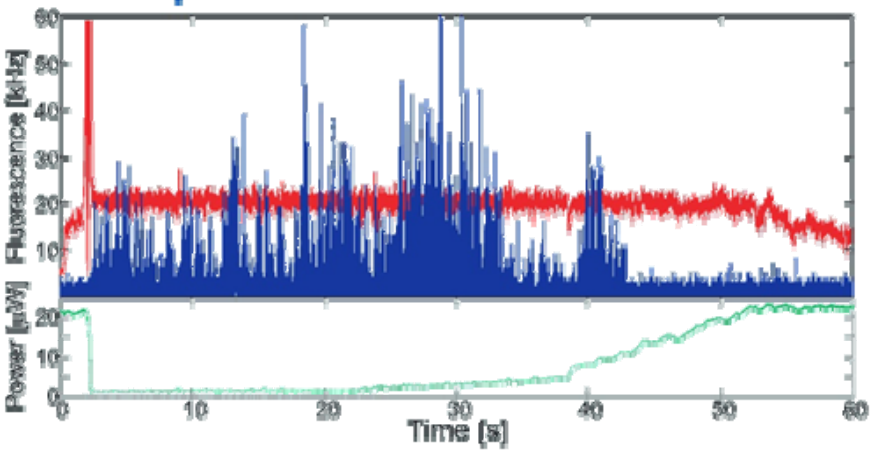


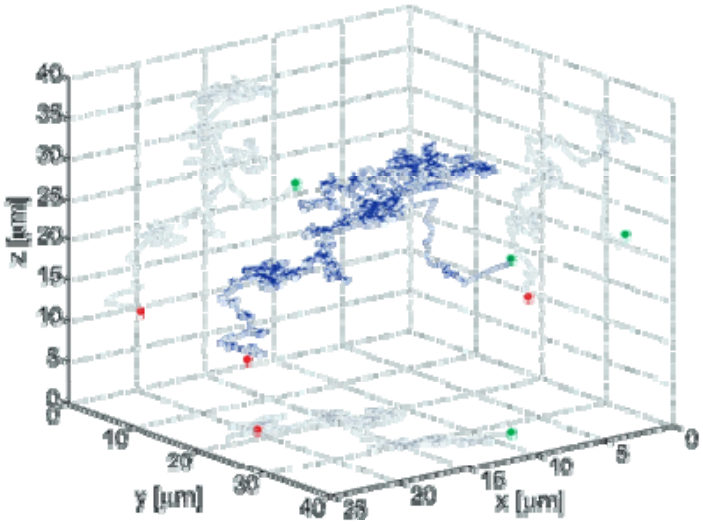
A. E. Cohen and W. E. Moerner, Phys. Rev. Lett. **98**, 116001 (2007)

Experiments on genomic lambda-DNA

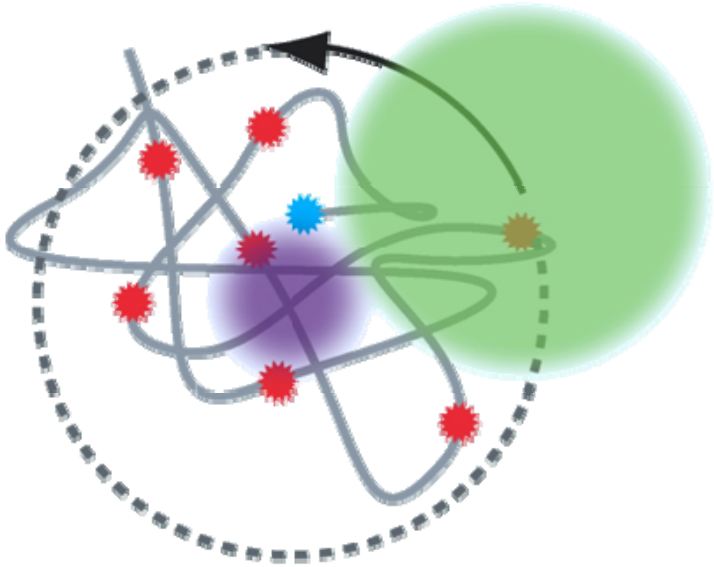
K. McHale and HM, submitted

Example data



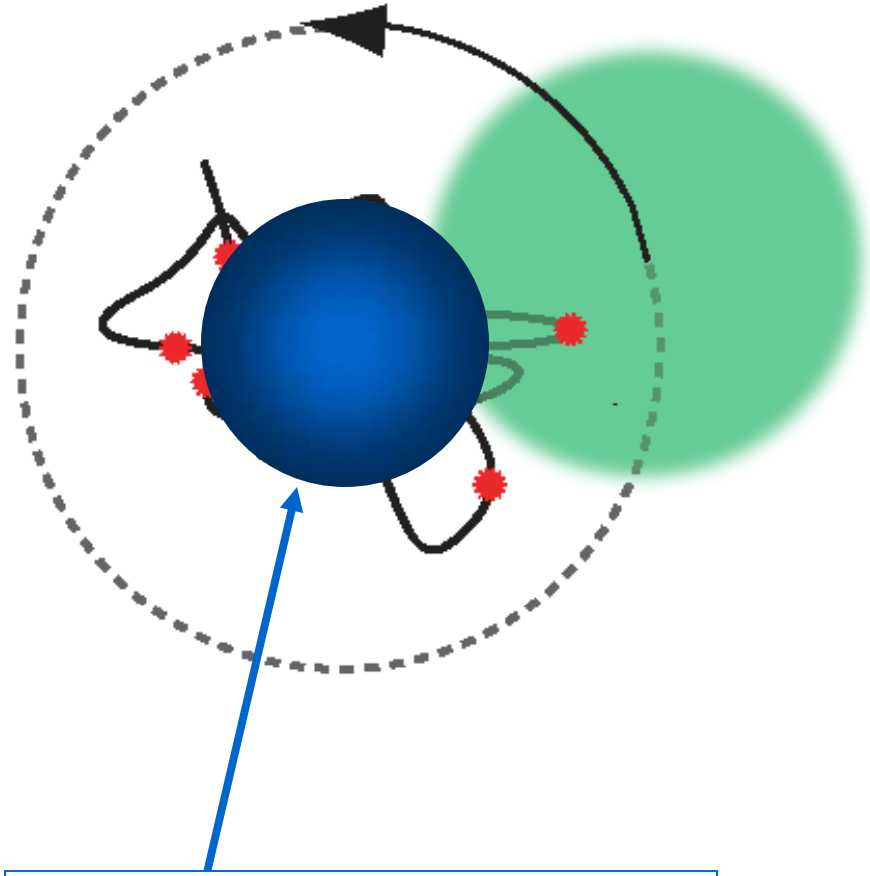


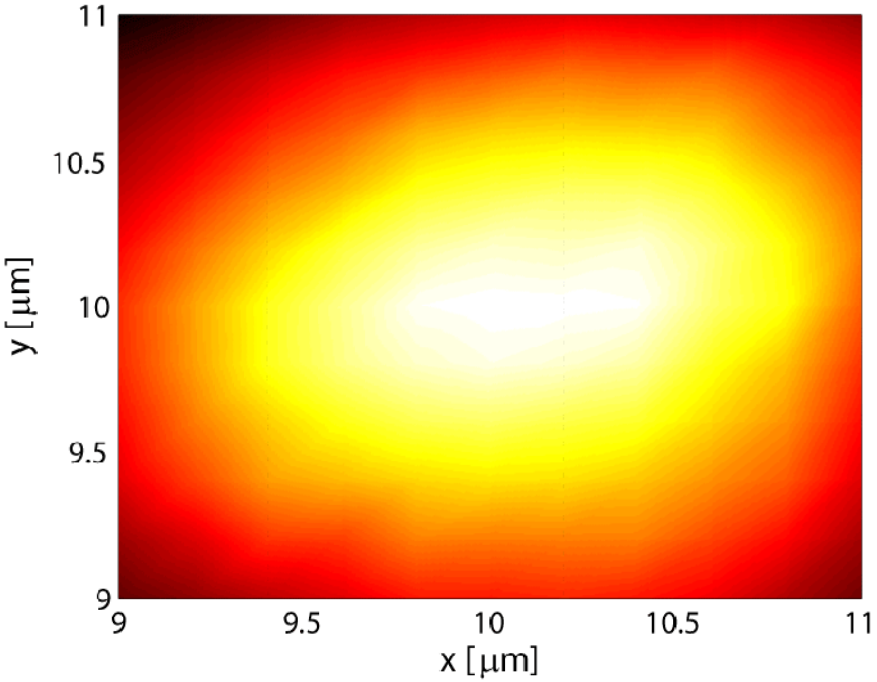
- Dense tracking labels (POPO-3)
- Single probe label (Atto425)
- Fluorescence rate and beam intensity stabilization
- Alternating excitation to prevent cross-talk

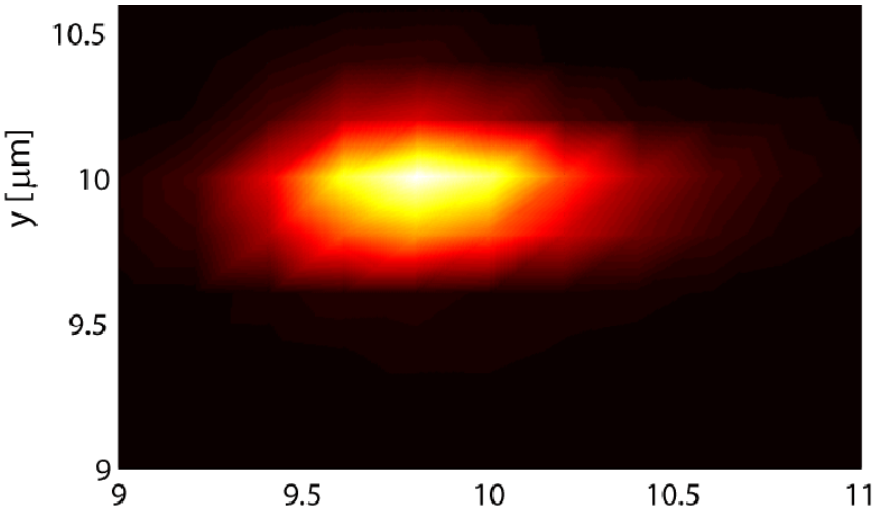


Two-color alternating excitation

K. McHale and HM, submitted





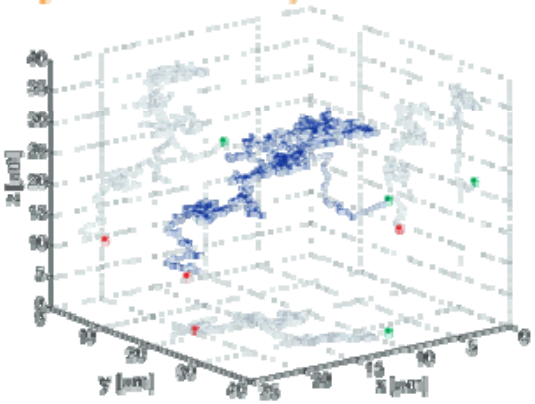


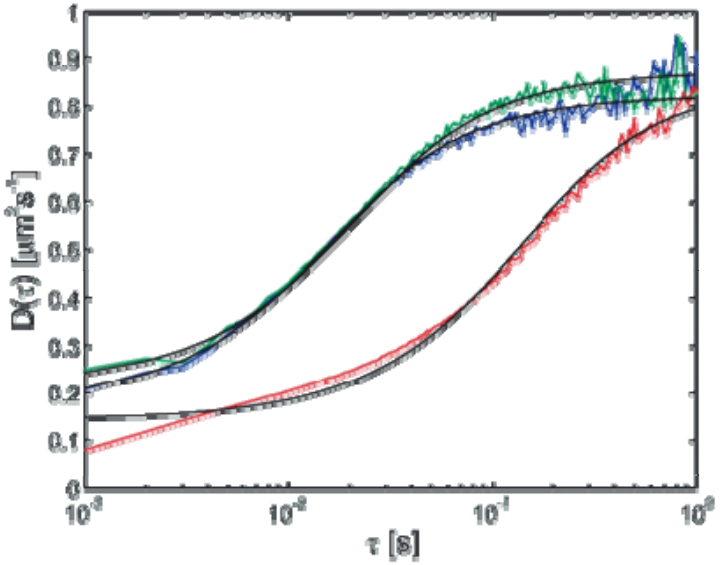
- improves FCS sensitivity
- decouples tracking from FCS
- could be used for FRET/catalysis

Center-of-mass motion

K. McHale and HM, submitted

Get systematic parameters and D from stage motion





Increment statistics:

$$\hat{D}(\tau) \equiv \frac{1}{2\tau}\left\langle (Y_{t+\tau} - Y_t)^2 \right\rangle$$

- Tracking bandwidths: $\gamma_{xy} = 15\text{Hz}$, $\gamma_z = 2\text{Hz}$
- RMS localization: $L_{xy} = 110\text{nm}$, $L_z = 290\text{nm}$
- Diffusion coefficient: $D = (0.80 \pm 0.05)\mu\text{m}^2/\text{s}$

D inconsistent with all other measurements!
(but really can't be wrong...)

Intramolecular motion

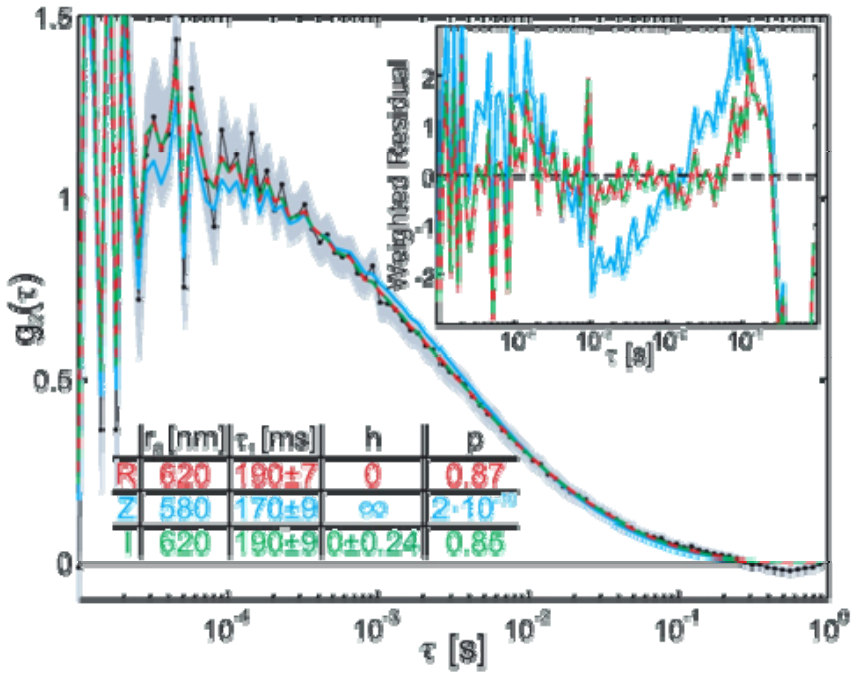
K. McHale and HM, submitted

$$\langle r_{t+\tau} \cdot r_t \rangle = \frac{6r_0^2}{\pi^2} \sum_{q=1}^{\infty} \frac{1}{q^2} e^{-\tau/\tau_q}$$

$$g_x(\tau) = \left[1 - \left(\frac{\sigma^2 e^{-\gamma\tau} + \frac{1}{3}\langle r_{t+\tau} \cdot r_t \rangle}{\sigma^2 + \frac{1}{3}r_0^2 + \frac{1}{4}w^2}\right)\right]^{-1/2}$$

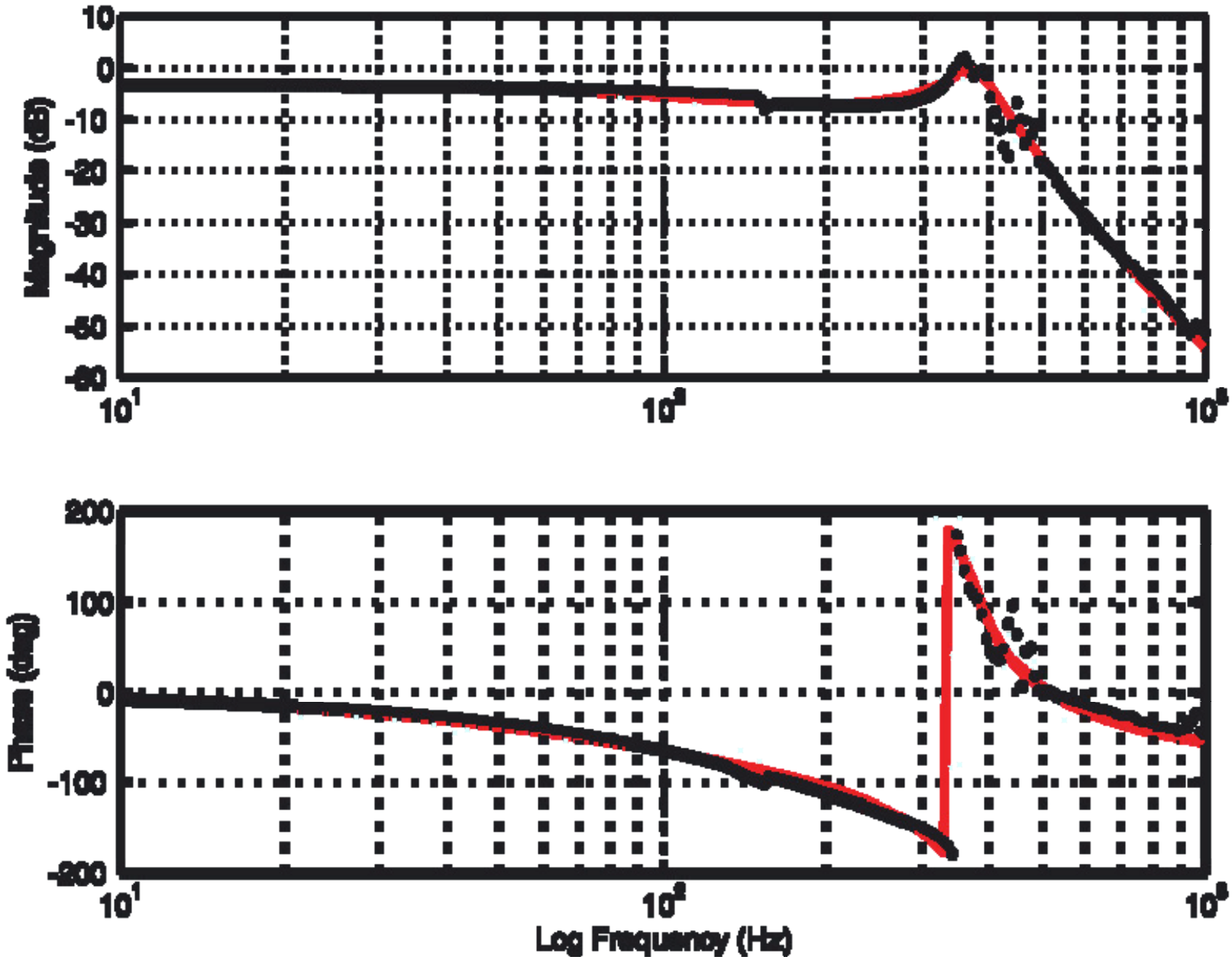
Fit parameters:

- r_0: size parameter ($R_g\sqrt{2}$)
- τ_1: characteristic time
- h: *draining* parameter



Interpretation

- (r_0, τ_1) theoretically consistent with $D \approx 0.8\mu m^2/s$
- Data and R/Z theories internally consistent to a factor of 2-4
- Zimm model is inconsistent with the FCS data!


10
0
-10
-20
-30
-40
-50
-60
Magnitude (dB)
10^1
10^2
10^3
200
100
0
-100
-200
Phase (deg)
10^1
10^2
10^3
Log Frequency (Hz)