# Diffusion and segmental dynamics of double-stranded DNA

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## Outline

Fluorescence correlation spectroscopy as an experimental tool to study diffusion and segmental dynamics of large macromolecules (including DNA)

Comparison of results for dsDNA fragments with predictions of semiflexible polymer theories

## Geometrical and mechanical properties of DNA:

Base-pair distance: 0.338 nm
Base-pairs per helical turn: 10.5
Persistence length: ~50 nm (in 0.1M NaCl)
Hydrated DNA thickness: ~2.5 nm

## Depending on the length, DNA can behave as stiff, semiflexible, or flexible polymer

Not much quantitative experimental data available

on diffusion and segmental dynamics of ds DNA

over the transition range from stiff to semiflexible chains

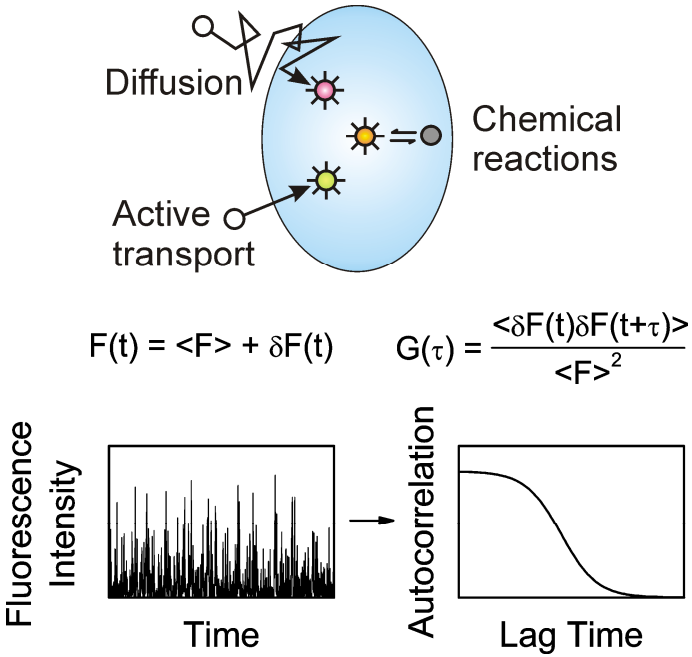
## Goals

Quantitative investigation of diffusion and intramolecular polymer dynamics of ds DNA over a wide range of lengths in the dilute regime

Based on quantitative data, establish the character of ds DNA polymer dynamics

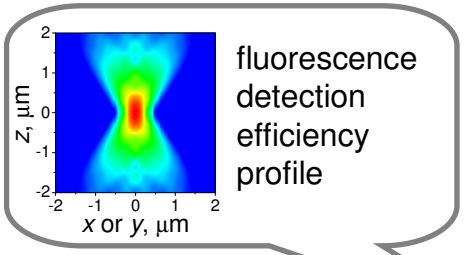
**Need a technique that would address mesoscopic polymer dynamics on a wide time range!**

# Fluorescence Correlation Spectroscopy: The basic idea




First introduced in the early 1970s
[Magde, Elson, Webb, *Phys. Rev. Lett.* **29** (1972) 705]

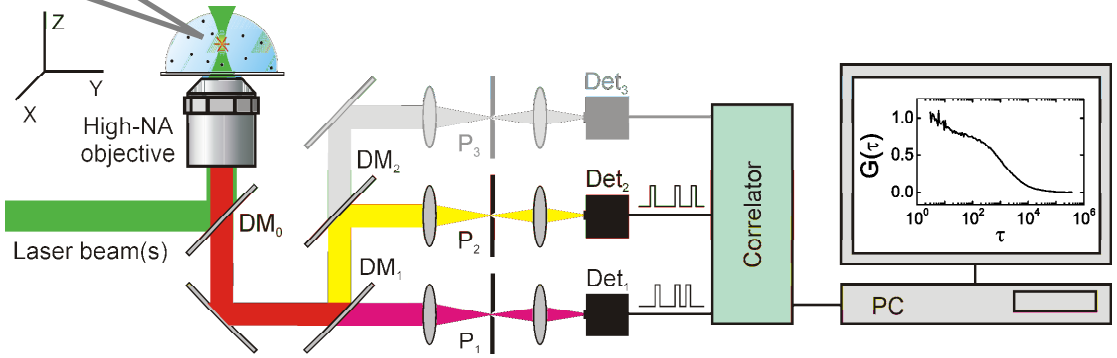
Revival and development since mid-1990s




## Typical experimental setup

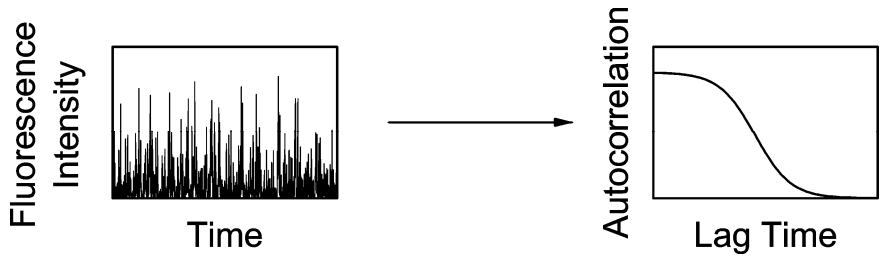
Confocal fluorescence detection by means of a high NA objective

Observation volumes of order of $1\mathrm{f}l = 10^{-15}l$



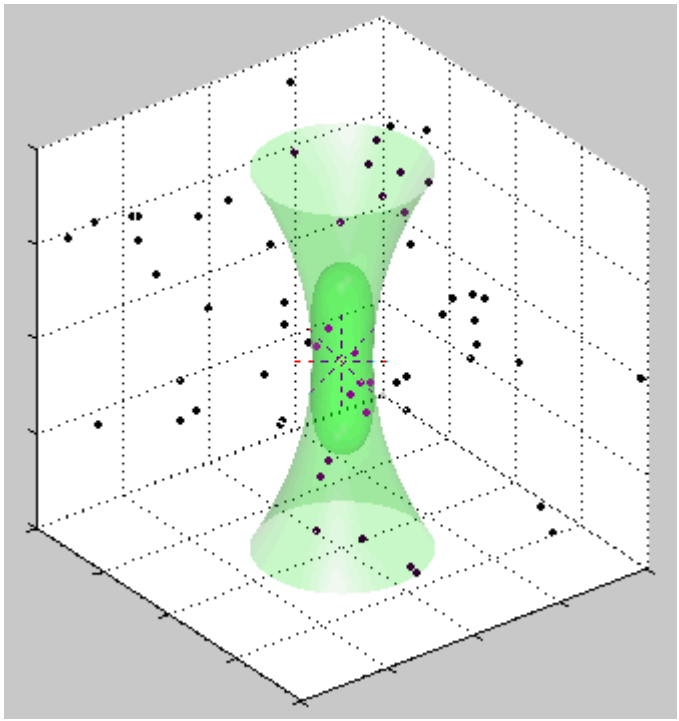

Tight focusing by a high-NA objective
+
Pinhole rejection of out-of-focus contributions
=
Small detection volume of order of 1fL

**Fluctuations in detected fluorescence signal are recorded and correlated**



## Origin of fluctuations in the fluorescence signal:

- Diffusional motion of fluorescently labeled particles in and out of an observation volume
  (typical applications)
- Motion of the fluorescent label due to internal degrees of freedom
  (our use of the technique)
- Fluctuations of molecular brightness
  (due to photophysical processes
  or chemical reactions in equilibrium)



**Experimental conditions necessary for pronounced fluorescence fluctuations**

Need to detect fluorescence from small ensembles of molecules

=> small detection volumes, low concentrations (~ $10^{-9}$ M),
close to single molecule per detection volume

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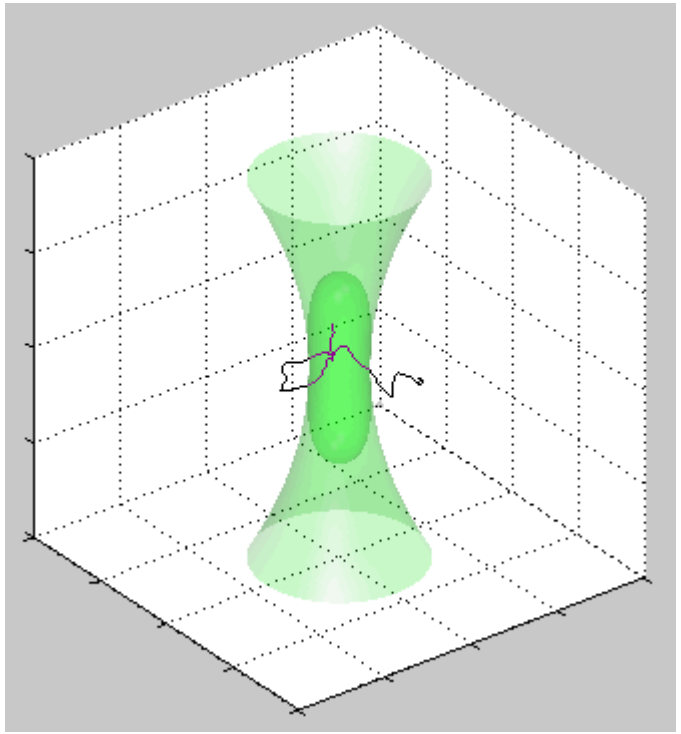


**Experimental conditions necessary for pronounced fluorescence fluctuations**

Need to detect fluorescence from small ensembles of molecules

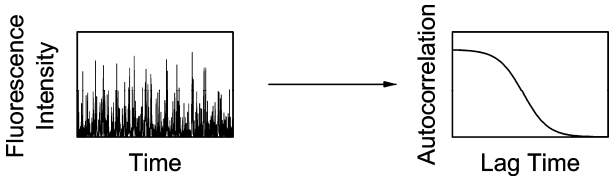
=> small detection volumes, low concentrations (~ $10^{-9}$ M),
close to single molecule per detection volume

FCS is applicable only to systems in thermodynamic equilibrium

Fluctuating fluorescence intensity $F(t)$ – stationary ergodic stochastic process

$$F(t) = \langle F \rangle + \delta F(t), \ \langle \delta F(t) \rangle = 0$$

## FCS autocorrelation function:




$$G(\tau) = \frac{\langle \delta F(t)\, \delta F(t+\tau) \rangle}{\langle F \rangle^2}$$

- Diffusion coefficient
- Intramolecular dynamics

In the absence of photophysical switching processes or chemical reactions:

$$G(\tau) = \int\int \Omega(\mathbf{r})\, \mathcal{G}(\mathbf{r}, \tau | \mathbf{r}', 0)\, \Omega(\mathbf{r}')\, d\mathbf{r} d\mathbf{r}' \Big/ \left[ \langle c \rangle \left( \int \Omega(\mathbf{r})\, d\mathbf{r} \right)^2 \right]$$

$\mathcal{G}(\mathbf{r}, t | \mathbf{r}', 0)$ – Green's function describing (stochastic) motion of the fluorescent particles

$\Omega(\mathbf{r})$ – fluorescence detection efficiency profile

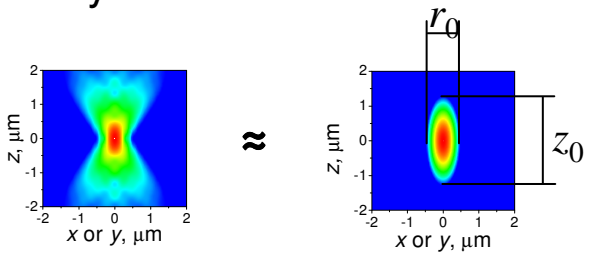
$\langle c \rangle$ – mean concentration of fluorescent particles

$$G(\infty) = 0; \ G(0) = 1/\langle N \rangle$$

$\langle N \rangle$ – effective number of molecules in detection volume

**Assumptions:**

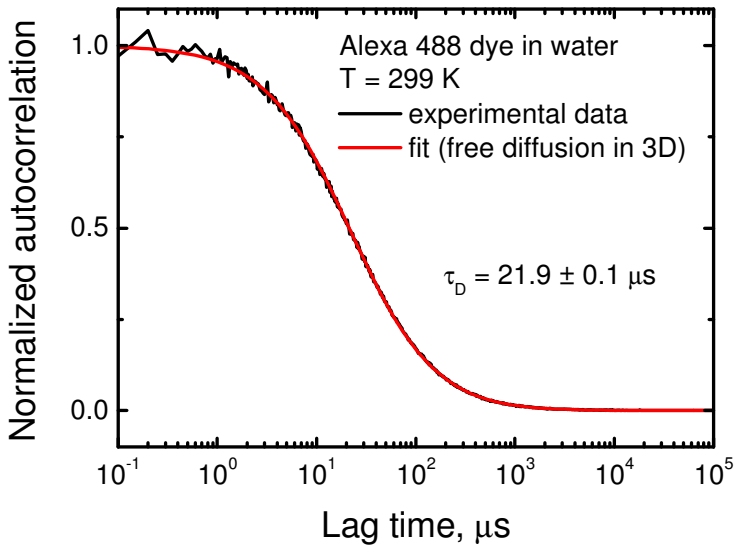
- Brownian motion of particles is unrestricted and described by a Gaussian Green's function
- Fluorescence detection efficiency profile is well approximated by a 3D Gaussian

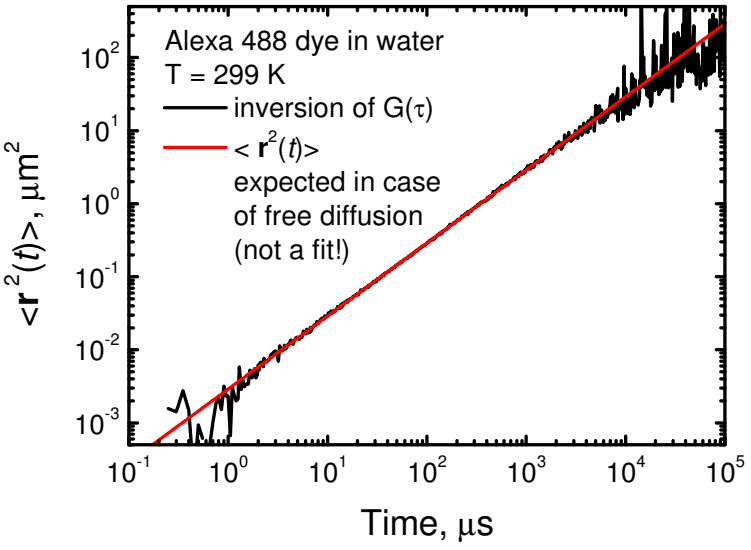



Then the mean square displacement can be obtained by inverting correlation data:

3D: $$G(\tau)/G(0) = \left(1+\tfrac{2}{3}\left\langle \mathbf{r}^2(t)\right\rangle / r_0^2\right)^{-1}\left(1+\tfrac{2}{3}\left\langle \mathbf{r}^2(t)\right\rangle / z_0^2\right)^{-1/2} \quad \rightarrow \quad MSD(t) = \left\langle \mathbf{r}^2(t)\right\rangle$$

Example: Fickian diffusion -- Freely diffusing dye in water

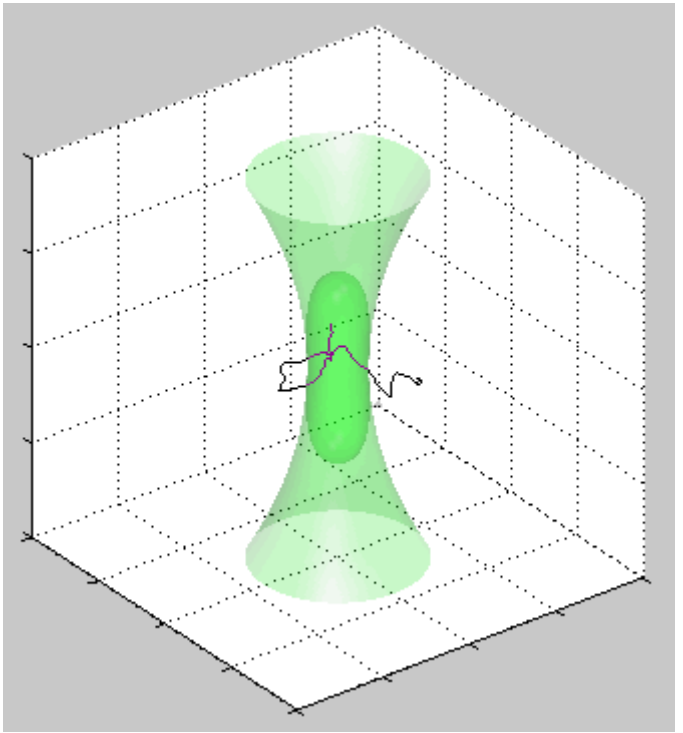


## Experimental approach:

Fluorescence Correlation Spectroscopy
of single-end fluorescence labeled DNA macromolecules

- No averaging of dynamics over the polymer molecule!
- Provides more detailed information on segmental dynamics than conventional techniques
  (Dynamic Light Scattering, Transient Electric Birefringence)

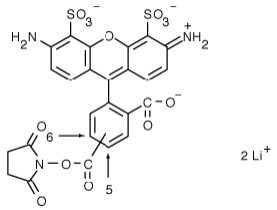


## Producing identical single-labeled ds-DNA fragments

Quantitative experiments on polymer dynamics require *well-defined monodisperse* samples covering a wide range of molecular weights

**Monodisperse single-end labeled dsDNA fragments were produced using the Polymerase Chain Reaction**

Alexa Fluor 488



Forward primer is fluorescently labeled

`AlexaFluor488 – C6 amino linker – 5′– GCG GCA TAT CAC AAA ACG – 3′`

Reverse primers determine the lengths of $\lambda$ -phage DNA fragments

As a result:

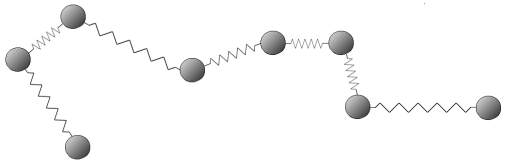
- identical
- monodisperse
- identically end-labeled

dsDNA fragments with a high purity

➔ dsDNA fragments in the length range of $10^2$…2 x $10^4$ bp

Additionally, single-end labeled straight DNA fragments with lengths of 15, 25, 40, and 70 bp were synthesized based on the designed sequences

# Generic bead & spring models



**Scaling dependences for polymer diffusion coefficient and relaxation time**

**Short-time MSD behavior of the end monomer**

## Rouse model

[P.E. Rouse, *J. Chem. Phys.* **21** (1953) 1272]

elastic forces between polymer segments
+ viscous drag

$$D \sim L^{-1}, \qquad \tau_1 \sim L^2$$

$$\left\langle \mathbf{r}^2(t) \right\rangle \sim t^{1/2}$$

## Zimm model

[B.H. Zimm, *J. Chem. Phys.* **24** (1956) 269]

Rouse model
+ hydrodynamic interactions between segments

$$D \sim L^{-1/2}, \qquad \tau_1 \sim L^{3/2}$$

($\theta$-conditions)

$$\left\langle \mathbf{r}^2(t) \right\rangle \sim t^{2/3}$$

## Semiflexible chains with hydrodynamic interactions

[L. Harnau, R. Winkler, P. Reineker *J.Chem.Phys.* **104** (1996) 6355]
[M. Hinczewski *et al.*, *Macromolecules* **42** (2009) 860]

chain persistence and elasticity
+ viscous drag
+ hydrodynamic interactions along the chain

No universal scaling

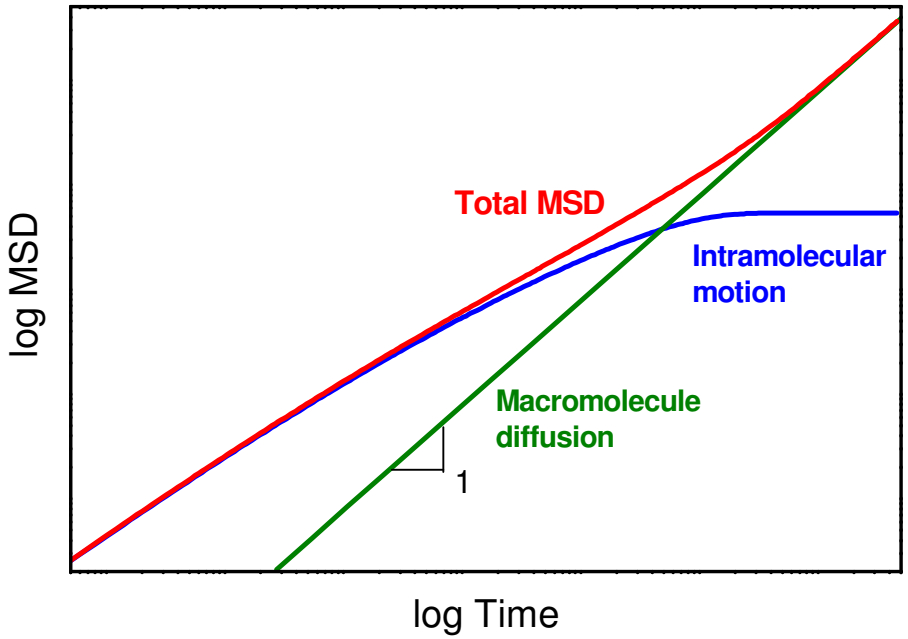
For very long chains ($L >> l_p$), shows Zimm-type behavior

$$\left\langle \mathbf{r}^2(t) \right\rangle \sim t^{3/4}$$

# MSD of the end monomer: Expected behavior

Short times: segmental dynamics of the polymer

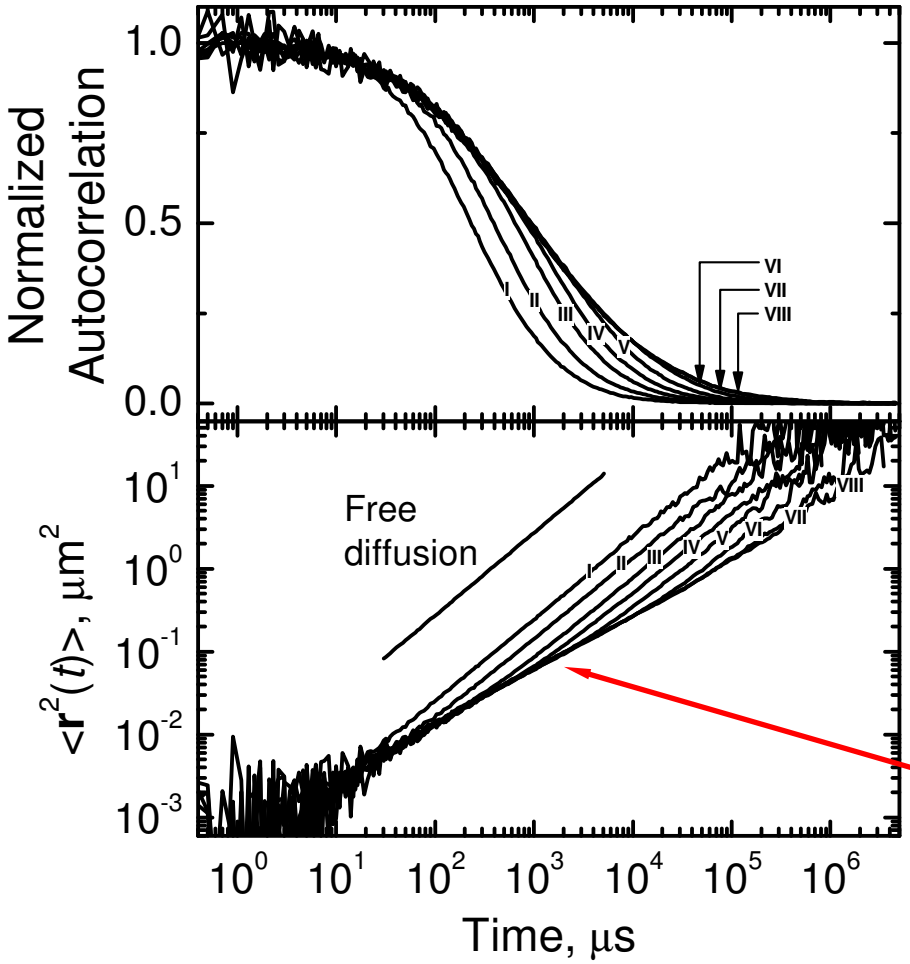
Long times: translational Brownian motion of the macromolecule




End monomer MSD = MSD_macromolecule + MSD_segmental_dynamics

# Evidence of DNA segmental dynamics from FCS data

Experiments on DNA fragments spanning more than two orders of magnitude in length




Single-end labeled $\lambda$-phage DNA fragments

| Fragment | Length | Contour length |
|---|---|---|
| I | 0.1 kbp | 0.034 $\mu$m |
| II | 0.2 kbp | 0.068 $\mu$m |
| III | 0.5 kbp | 0.17 $\mu$m |
| IV | 1 kbp | 0.34 $\mu$m |
| V | 2 kbp | 0.68 $\mu$m |
| VI | 5 kbp | 1.7 $\mu$m |
| VII | 10 kbp | 3.4 $\mu$m |
| VIII | 20 kbp | 6.8 $\mu$m |

Universal time dependence due to segmental dynamics of DNA molecules

E.P. Petrov, T. Ohrt, R.G. Winkler, P. Schwille
*Phys.Rev.Lett* **97** (2006) 258101

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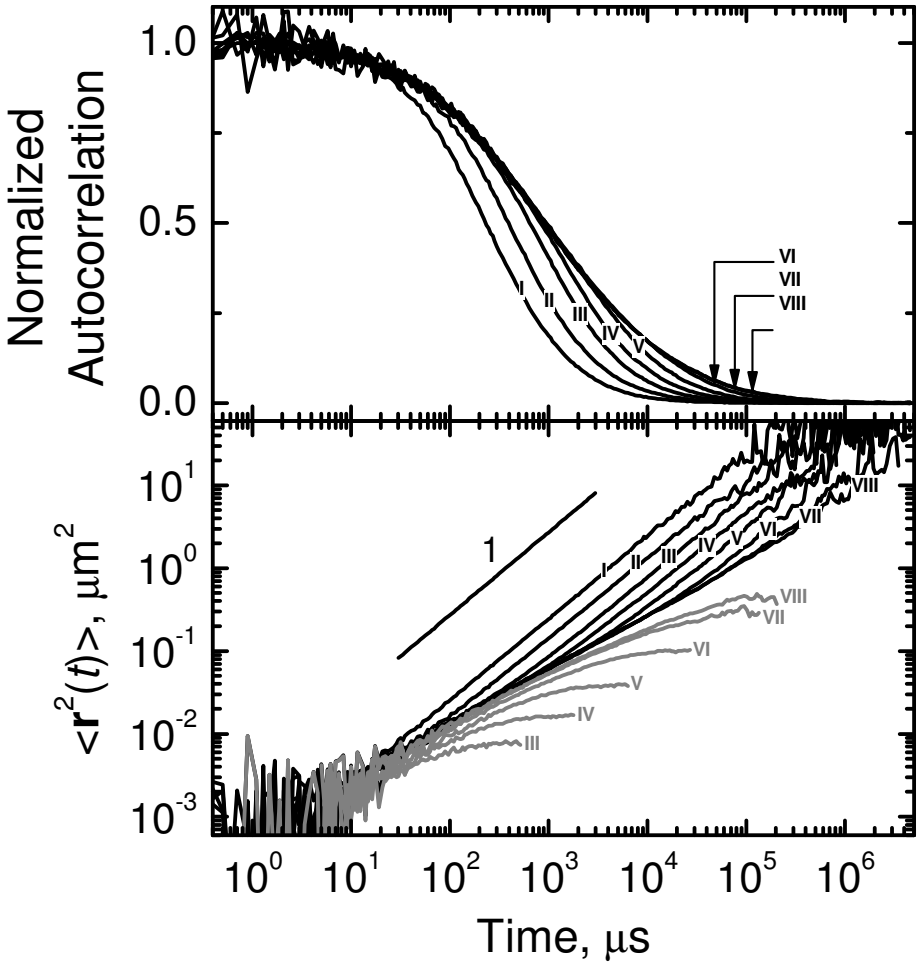


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Even for longest fragments,
end monomer dynamics shows saturation.
Straightforward application of power law analysis is impossible

## Evidence of DNA segmental dynamics from FCS data

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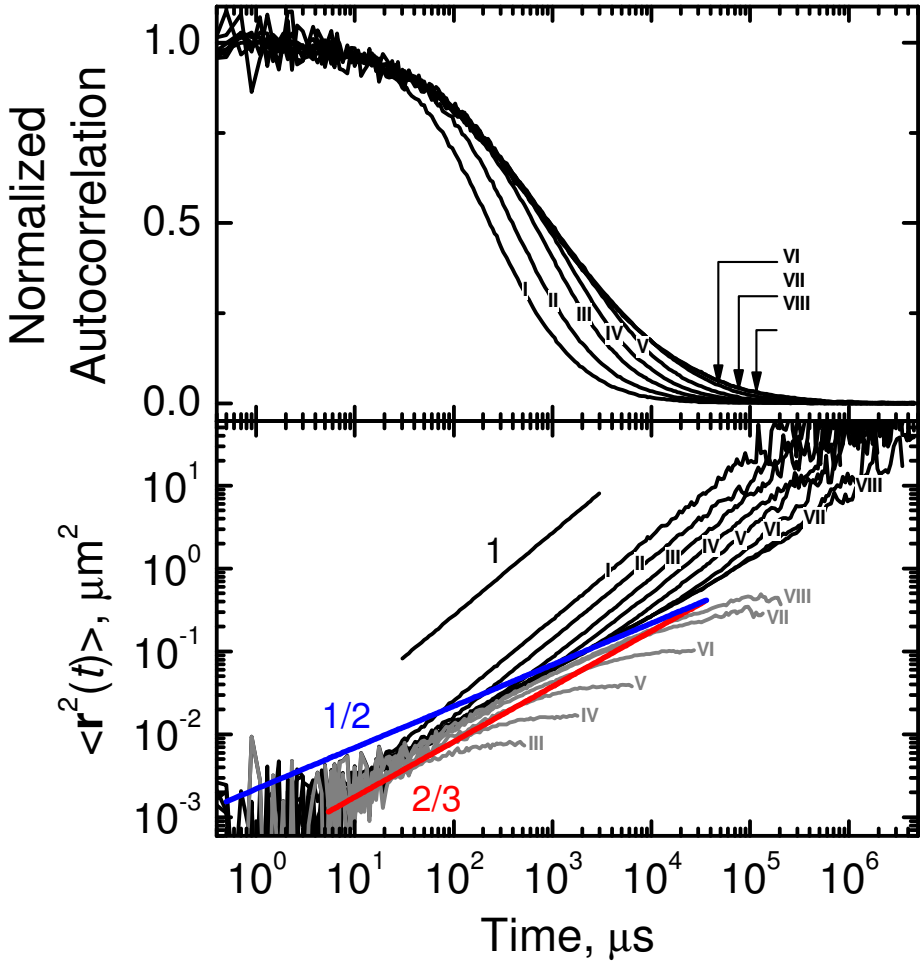


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…though the Rouse dynamics ( ~ $t^{1/2}$ )
can be immediately ruled out
(as expected)

## Quantitative analysis of FCS correlation functions

Harnau-Winkler-Reineker (HWR) model

[L. Harnau, R. Winkler, P. Reineker *J.Chem.Phys.* **104** (1996) 6355]

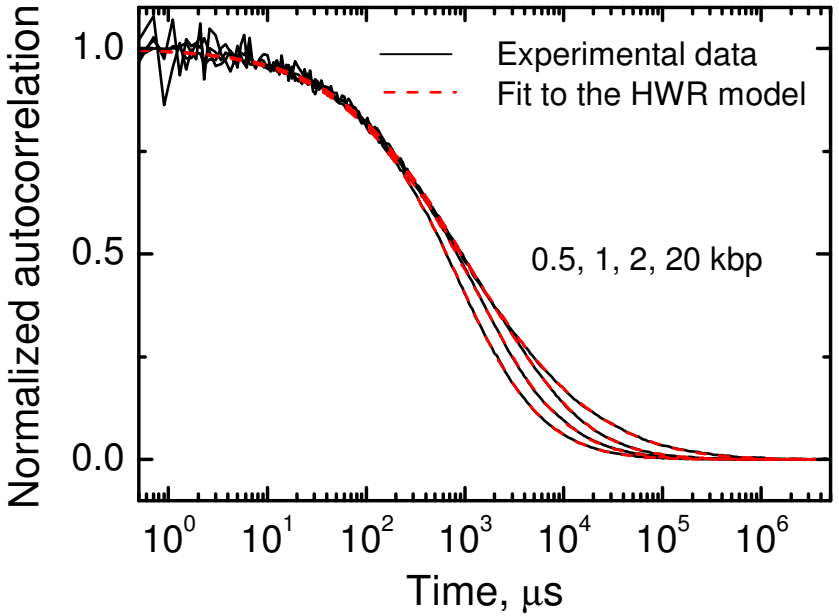
$$\left\langle \mathbf{r}^2(t) \right\rangle = 6Dt + \frac{2k_BT}{\pi\eta} \sum_{l=1}^{\infty} \tau_l \psi_l^2 (L/2)\left(1 - \exp\left(-t/\tilde{\tau}_l\right)\right)$$

$$\tilde{\tau}_l = \tau_l / \left(1 + 3\pi\eta\Lambda H_{ll}\right), \qquad D = k_BT\left(1 + \Lambda_D H_{00}\right)/3\pi\eta L$$

To achieve complete *quantitative* agreement, slight modification of theory was required: $\Lambda \approx 0.6, \quad \Lambda_D \approx 0.9$

(which is OK taking into account the simplicity of the theory and approximations involved)

E.P. Petrov, T. Ohrt, R.G. Winkler, P. Schwille *Phys.Rev.Lett* **97** (2006) 258101

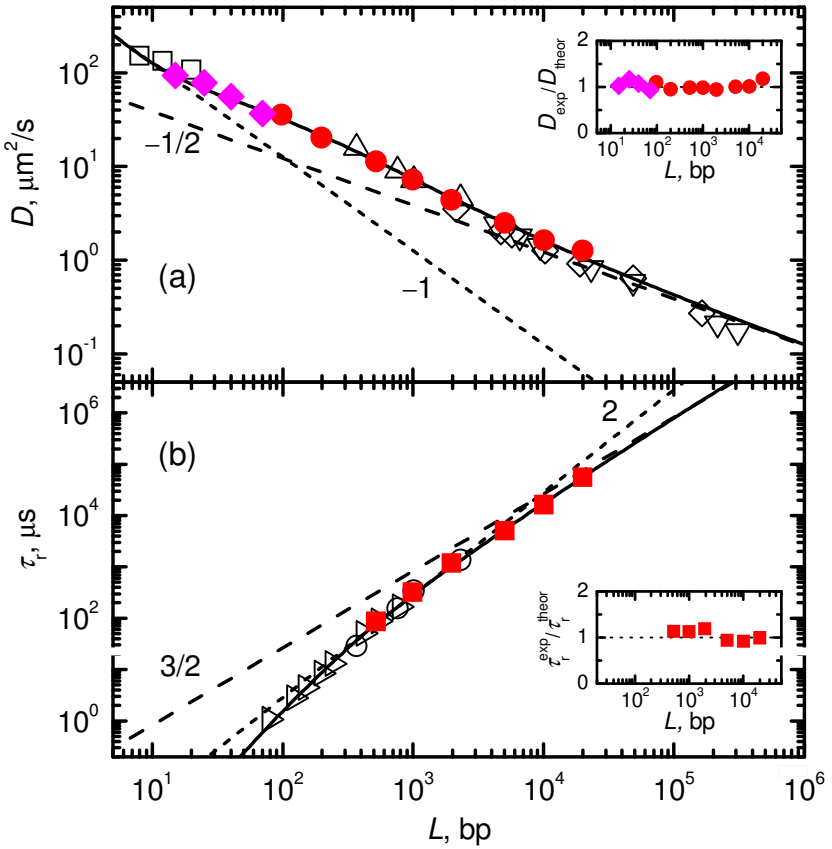



## Model-based data analysis

**Parameters obtained simultaneously from data:**

- diffusion coefficient (model-independent!)
- longest relaxation time

## Scaling behavior of $D$ and $\tau$




1. Comparison with generic Zimm and Rouse models. No agreement! (as expected)
2. Comparison with experimental data on $D$ and $\tau$ obtained *separately* by *different* techniques. Excellent agreement!
3. Comparison with the semiflexible polymer theory. [L. Harnau, R. Winkler, P. Reineker *J.Chem.Phys.* **104** (1996) 6355] Excellent agreement!

E.P. Petrov, T. Ohrt, R.G. Winkler, P. Schwille
*Phys.Rev.Lett* **97** (2006) 258101

● ■ ◆ Our FCS-based results

**Data obtained by different techniques:**

**Diffusion coefficient:**

□, △ dynamic light scattering [W. Eimer and R. Pecora, *J. Chem. Phys.* **94**, 2324 (1991), S.S. Sorlie and R. Pecora, *Macromolecules* **23**(1990)487.]
▽ single-molecule fluorescence microscopy [D.E. Smith, T.T. Perkins, and S. Chu, *Macromolecules* **29**(1996)1372]
◇ electrophoresis [A.E. Nkodo, J.M. Garnier, B. Tinland, H. Ren *et al.*, *Electrophoresis* **22**(2001)2424]

**Polymer relaxation time:**

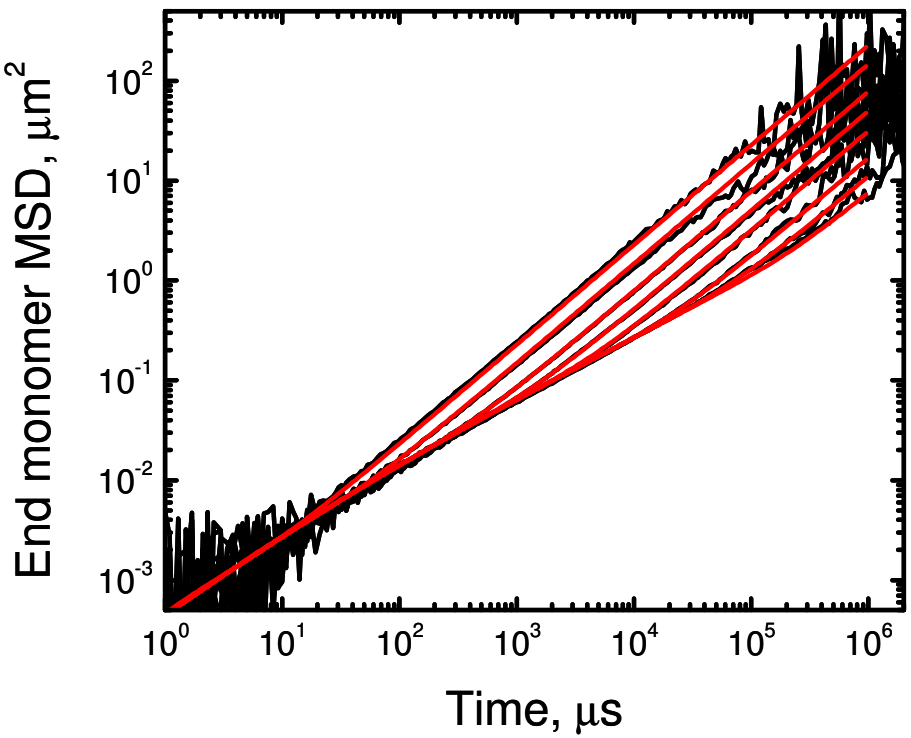
○, ▷ transient electric birefringence [R.J. Lewis, R. Pecora, D. Eden, *Macromolecules* **19**(1986)134; Y. Lu, B. Weers,N.C. Stellwagen, *Biopolymers* **61**(2001)261]

## Comparison with the new theory

New dynamic mean-field with much more rigorous account for hydrodynamic interactions

M. Hinczewski *et al.*, *Macromolecules* **42** (2009) 860

The only parameters of the theory are DNA persistence length and radius




Our experimental data

Hinczewski & Netz, MFT computations

$l_p$ = 50 nm, $a$ = 1 nm

**No fitting!**

## Conclusions

1. Fluorescence Correlation Spectroscopy is established as a quantitative experimental technique in polymer physics allowing simultaneous investigation of diffusion and segmental dynamics of polymer

2. Double-stranded DNA behaves as a semiflexible polymer with strong hydrodynamic interactions

3. Excellent agreement with the semiflexible polymer theories for DNA fragment lengths 100…2 x$10^4$ bp

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