Tracking Fluorescence Correlation Spectroscopy of individual biomolecules

Kevin McHale¹, Andrew Berglund², Mike Zhang, Charles Limouse, Chandra Raman³, Hideo Mabuchi

Edward L. Ginzton Laboratory, Stanford University

¹Chemical Physics Laboratory, NIDDK, NIH ²CNST Nanofabrication lab, NIST ³Physics, 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

High FRET efficiency

Low 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


Donor autocorrelation

Acceptor autocorrelation

Cross-correlation

Intermittent FRET rate reproduces qualitative features

~ 5 ns
Photon-stream filtering: dynamics vs. shot-noise

\[ p ( \tilde{\theta} | \Xi[0, t]) = q (\Xi(t) | \tilde{\theta}) \ p (\tilde{\theta} | \Xi[0, t]) \]

\[ d \tilde{\theta}_t = A_t \tilde{\theta}_t \ dt + \kappa_t [d \Xi_t - C \tilde{\theta}_t \ dt] \]
Near axis of rotation the demodulated fluorescence provides a linear error signal.
Cohen & Moerner: anti-Brownian electrokinetic trap


Tracking data channel(s)


- no particle in focus; background photons only
- tracking a 60nm bead; more “diffusive” trajectory
- tracking a 210 nm bead; less diffusive trajectory

Counts/ms

Position [um]

Time [s]

small bead requires higher excitation power for same count rate
Tracking performance near the shotnoise limit (2D)

Fast classification of individual nano-particles (beads)


90% confidence with only ~600 photons!
3D Feedback tracking microscopy

Schematic apparatus

Free-solution dynamics of large dsDNA molecules

A. J. Spakowitz, Stanford University

www.sciencelearn.org.nz

“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
- DNA in ABEL trap: Cohen & Moerner, PRL and PNAS, 2007

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

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

$D$ inconsistent with all other measurements!
(but really can’t be wrong...)
Intramolecular motion

K. McHale and HM, submitted

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

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

Fit parameters:

- \( r_0 \): size parameter (\( R_g \sqrt{2} \))
- \( \tau_1 \): characteristic time
- \( h \): draining parameter

Interpretation

- \((r_0, \tau_1)\) theoretically consistent with \( D \approx 0.8 \mu\text{m}^2/\text{s} \)
- Data and R/Z theories internally consistent to a factor of 2-4
- Zimm model is inconsistent with the FCS data!