Single Molecule Bio-Physics
Single Molecule Fluorescence Techniques

1. Filter barrier filter: lets through only blue light with a wavelength between 450 and 495 nm.

2. Beam-splitting mirror: reflects light below 510 nm but transmits light above 510 nm.

3. Second barrier filter: cuts out unwanted fluorescent signals, passing the specific green fluorescence emission between 530 and 560 nm.

Transmission (%)

Wavelength (nm)
Single Molecule Fluorescence Techniques

State of the Art imaging of single (immobilized) fluorescent Cy5 molecules
Green Fluorescent Protein (GFP)
Discovered in Jelly Fish
Nobel Prize 2008
Super-Resolution Microscopy
Super-Resolution Microscopy

4 s movie of actin labeled Cy5 molecules under 100 µM AA –O₂
1 ms integration time

Real-time movie

Analyzing frame by frame

Pictures: Tinnefeld Lab
Super-Resolution Microscopy

Actin Fibers stained with ATTO647

Pictures: Sauer Lab
Fret / Quenching

High FRET

Unfolding

Refolding

Low FRET
Force Spectroscopy
DNA Force Extension by Magnetic Tweezers

[Diagram showing DNA molecule being extended by magnetic tweezers, with a graph showing force versus extension with different models for comparison.]
Applying force to single molecules
Molecular function of muscle
Estimation of entropic forces on a polymer
a) - ATP

b) + ATP (2mM)
Force Spectroscopy with Optical Tweezers
Optical Tweezers


Mie-Regime: Particle $\gg \lambda$ : ray-optics

Rayleigh-Regime
Particle diameter $\ll \lambda$
Consider particle as electric dipole

Typical Trapping wavelength: 1064 nm
Investigation of Kinesin
Light Driven Microfluidics
Lab-on-a-Chip

Controlled Fluid Flow without channels?
Full Fluid Control

100 µm
Setup

Fluorescence Microscope

IR Laser @ 1450 nm

x-y-scanner
What is the driving mechanism?
Moving warm spot

Spot

Expansion  Contraction

Viscosity  Temperature

Viscosity  Temperature
Finite Element Analysis

\[-\rho \frac{\partial u}{\partial t} - \rho (u u_x + v u_y + w u_z) = \frac{\partial}{\partial x} [p - 2 \eta u_x] - \frac{\partial}{\partial y} [\eta (u_y + v_x)] - \frac{\partial}{\partial z} [\eta (u_z + w_x)]\]

\[-\rho \frac{\partial v}{\partial t} - \rho (u v_x + v v_y + w v_z) = -\frac{\partial}{\partial x} [\eta (v_x + u_y)] + \frac{\partial}{\partial y} [p - 2 \eta v_y] - \frac{\partial}{\partial z} [\eta (v_z + w_y)]\]

\[-\rho \frac{\partial w}{\partial t} - \rho (u w_x + v w_y + w w_z) = -\frac{\partial}{\partial x} [\eta (w_x + u_z)] - \frac{\partial}{\partial y} [\eta (w_y + v_z)] + \frac{\partial}{\partial z} [p - 2 \eta w_z]\]

\[\frac{\partial \rho}{\partial t} + \rho (u u_x + v u_y + w u_z) + \rho_x u + \rho_y v + \rho_z w = 0\]
Temperature Imaging

- Relative Fluorescence [%]
- Temperature [K]

Graphs showing temperature and relative fluorescence.
Dependencies

\[ v \propto f \]

\[ v \propto \alpha \Delta T \times \beta \Delta T \propto \Delta T^2 \]

\[ v_{\text{pump}} = \frac{-3 \sqrt{\pi}}{4} \, f \alpha \beta b \Delta T^2 \]
Expansion coefficient and viscosity

\[ v \propto \alpha \beta \]

\[
\nu_{\text{pump}} = -\frac{3 \sqrt{\pi}}{4} f \alpha \beta b \Delta T^2
\]
More Efficient towards Nanofluidics

\[ v \propto \frac{1}{d^2} \]

Microfluidics in Gels
Pumping in Ice
Pumping in Ice

\[ v_{\text{pump}} \approx 0.14 \, d_{\text{spot}} \, f \]

Thermophoresis

\[ v = -D_T \nabla T \]

\[ \frac{c}{c_0} = \exp(-S_T \Delta T) \]

\[ D_T: \text{Thermodiffusion Coefficient} \]

\[ S_T = \frac{D_T}{D}: \text{Soret Coefficient} \]
Towards a Molecule Trap
Towards Accumulation

Thermogravitational Separation Column

\[
\frac{c_{\text{bottom}}}{c_{\text{top}}} = 2\times
\]

\[
\frac{c_{\text{bottom}}}{c_{\text{top}}} = \exp\left[0.42 \times S_T \times \Delta T \times \frac{\text{length}}{\text{width}}\right]
\]

<table>
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<tr>
<th>Aspect Ratio</th>
<th>10:1</th>
<th>50:1</th>
<th>125:1</th>
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<tr>
<td>Accumulation</td>
<td>7x</td>
<td>13,000x</td>
<td>10^{10}x</td>
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Concentration Problem at the Origin of Life


Problem for Applications: long equilibration times ~ hours/days
Linear Clusius Tube
Temperature Gradient & Bidirectional Flow

- Sapphire
- Water
- 50 nm Cr
- Silicon
- MOVING WARM SPOT
- COLD
- 2 µm
- 35 µm

- XY Mirrors
- IR Laser
- Laser Pump
- Backflow
Biderectinal Flow
Thermophoresis + Bidirectional Flow = Accumulation
Accumulation of 5 base single stranded DNA
Simulation of 50 base ss DNA

Finite Element Simulation

Concentration [fold]

Radius [μm]

Finite Element Simulation

Trapping Potential Depth [kT]

Measurement

Simulation

ss DNA length [bases]

Center Concentration [fold]

Measurement

Simulation

50 base DNA, single stranded

Time [s]
Vacuum Cleaner for 40nm beads

(real time)
Vacuum Cleaner for ss 50 base DNA
40nm bead trap

Polystyrene Spheres D = 40 nm, S_T = 0.04 1/K
Microfluidics in Ice
Parabolic Backflow

Asymmetric Pump

Parabolic Backflow