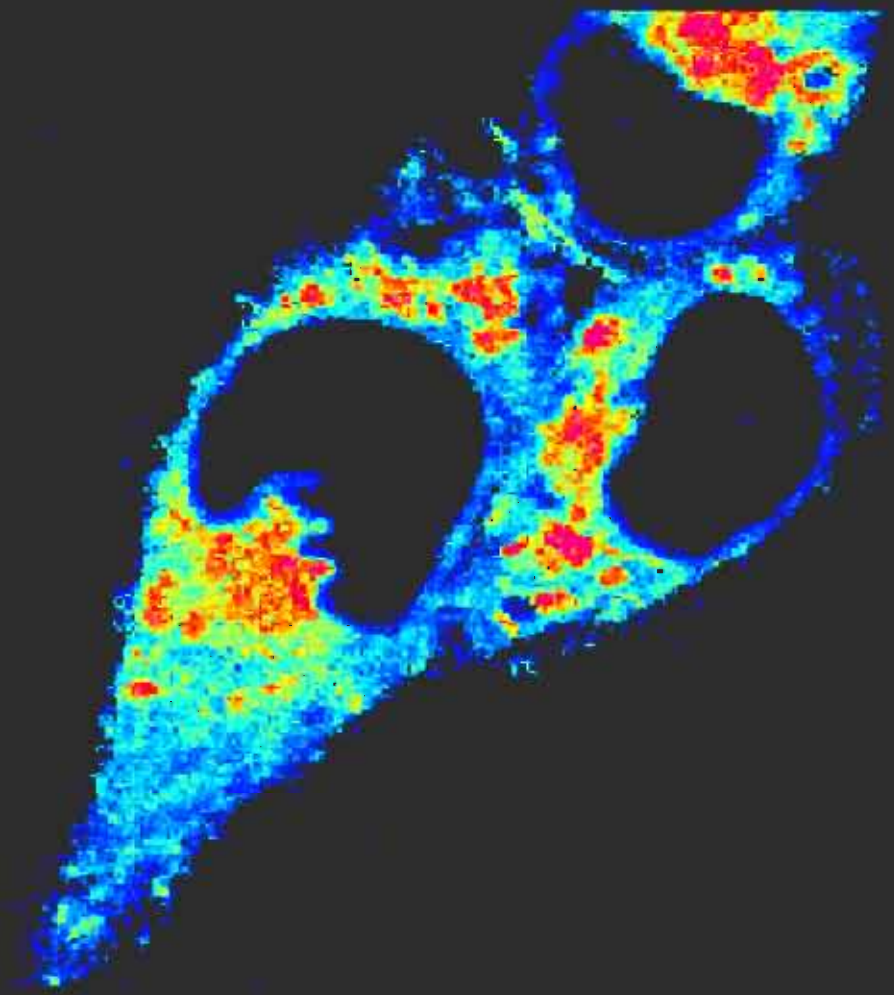
Peeking and Poking at Molecular Dynamics in Cells

Peter T. C. So



Mouse Fibroblast Cells Labeled With Membrane Fluidity Probe: Laurdan

OUTLINE

I. Molecular dynamics: diffusion, constrained diffusion, anomalous diffusion, directed motion

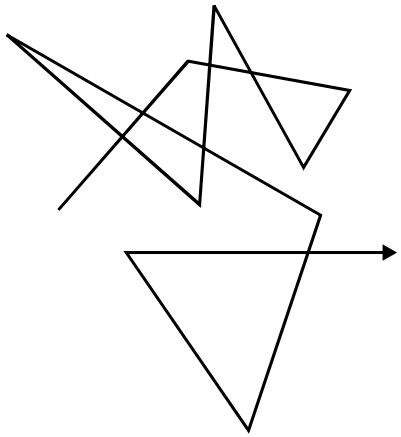
II. Peeking
- A. FRAP/PAF
- B. Time-resolved polarization
- C. FCS
- D. SPT
- E. LTM

III. Poking
- A. Optical micromanipulation
- B. Magnetic micromanipulation
- C. Manipulation of single proteins

Molecular Dynamic Processes

Molecular motion governed by only stochastic processes:

$$\frac{\partial C(\vec{r},t)}{\partial t} = D\nabla^2 C(\vec{r},t)$$

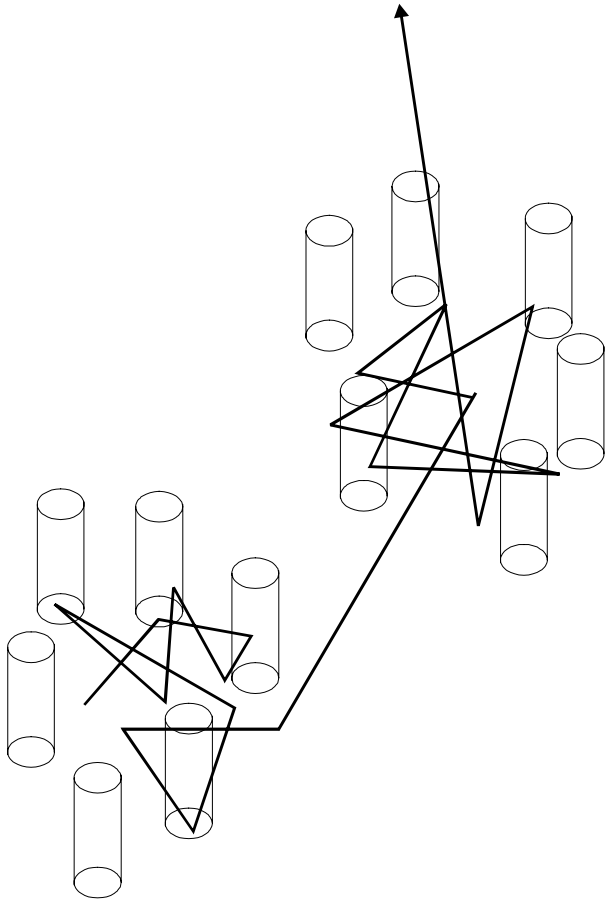


$$< |r|^2 > \propto Dt$$

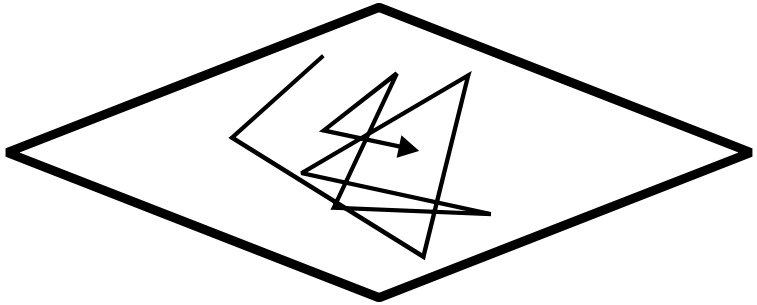
Molecular motion modified by the underlying structure and machinery

$$< |r|^2 > \propto Dt^{\alpha}$$

$$\alpha < 1$$

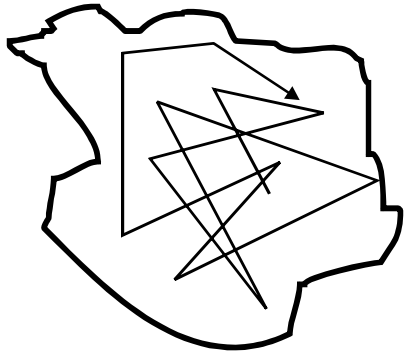


Molecular motion confined by the underlying structure

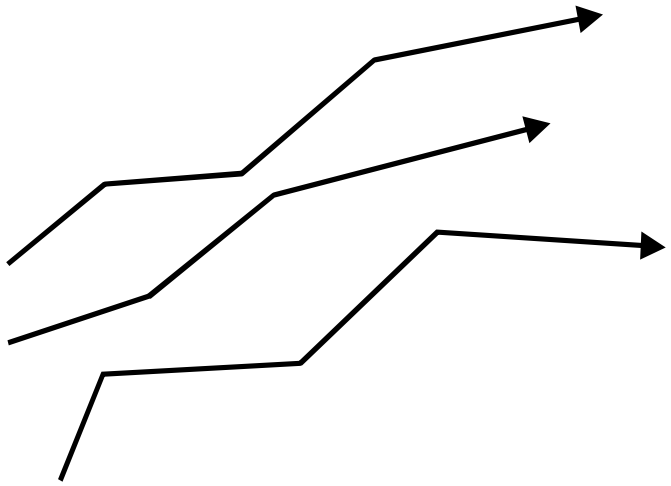


$$< |r|^2 > \propto Dt^{\alpha}$$ for small t

$$< |r|^2 > < r_0^2$$ For large t



Molecular motion driven by cellular motor proteins as well as stochastic processes

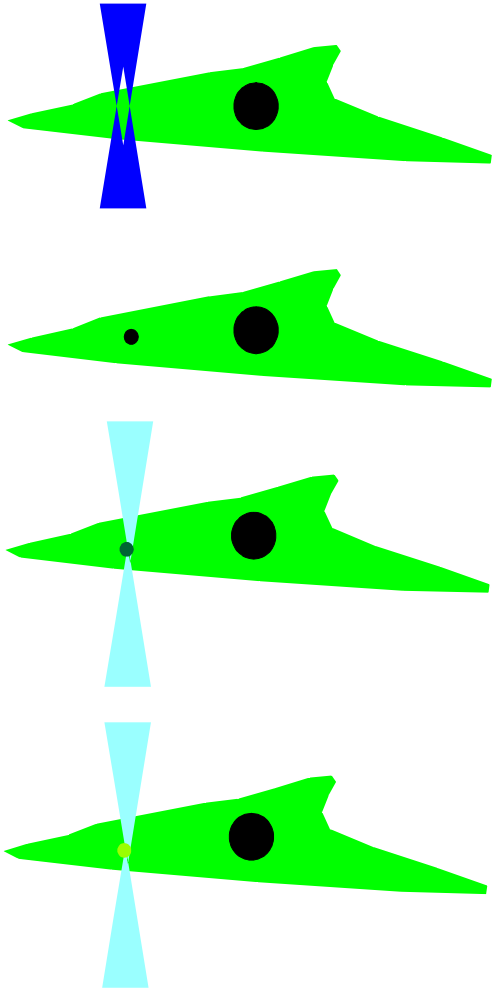


Fluorescence Recovery After Photobleaching (FRAP)

Photoactivation of Fluorescence (PAF)

FRAP

Basic idea:
Get rid of some fluorophores inside a pre-defined volume and watch the fluorescence come back.

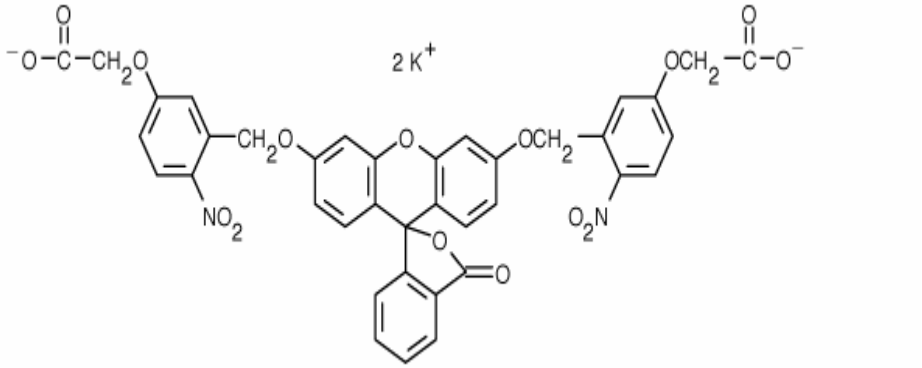


How to get rid of the fluorohpores?
Photobleaching-- photochemical destruction of the fluorophore:

- fluorescein : 10^3
- rhodamine: 10^6

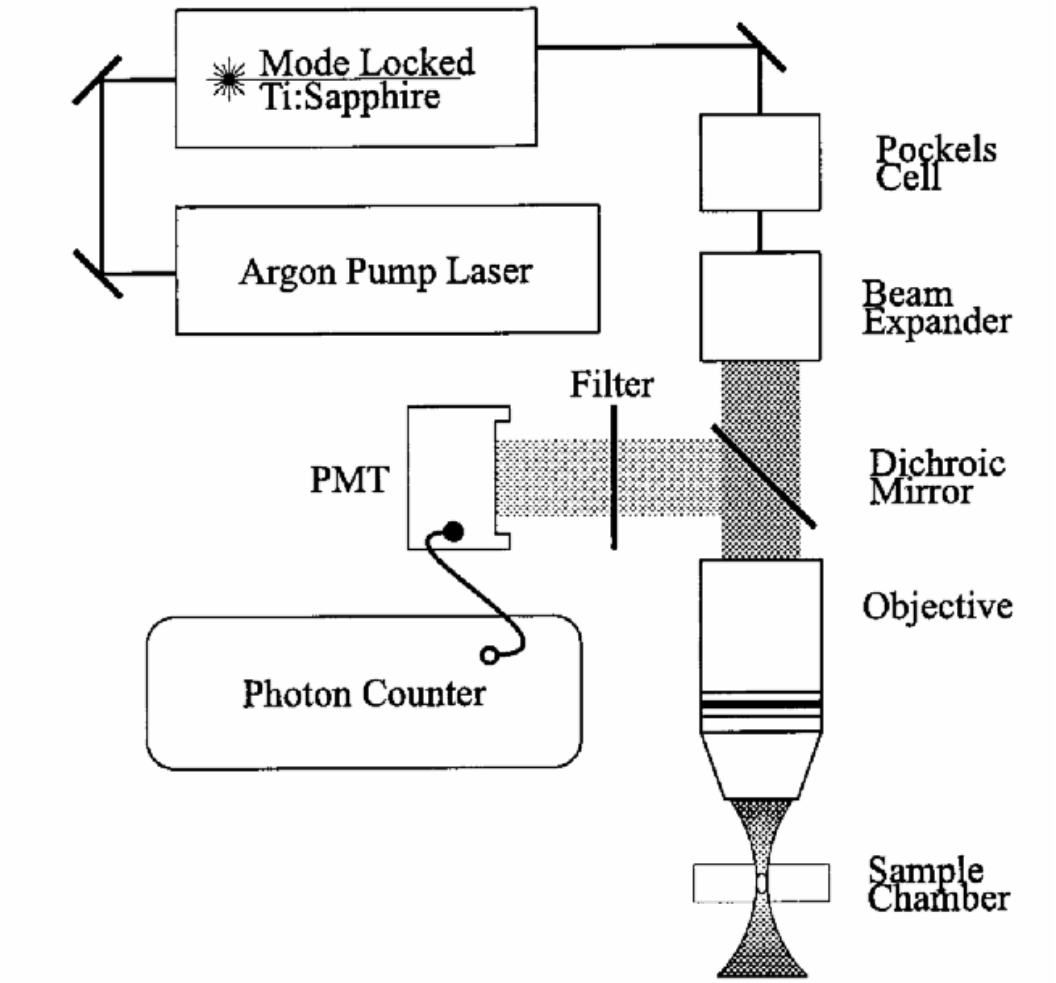
PAF:

Basic idea:
Reverse FRAP.
Create active fluorophores
inside a well confined area and
watch them diffuse out.



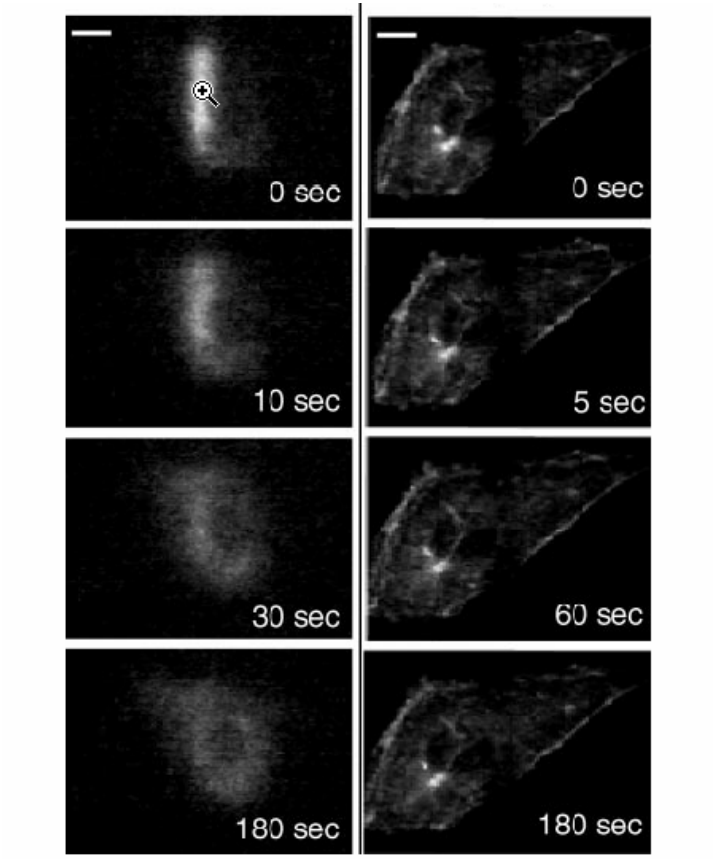
How to “make” new fluorophores?
Use caged fluorescent molecule --
molecule that has a “caging” group
that quenches the fluorophore until the
cage group is removed by photochemical
process.

Typical instrument arrangement for FRAP/PAF experiment



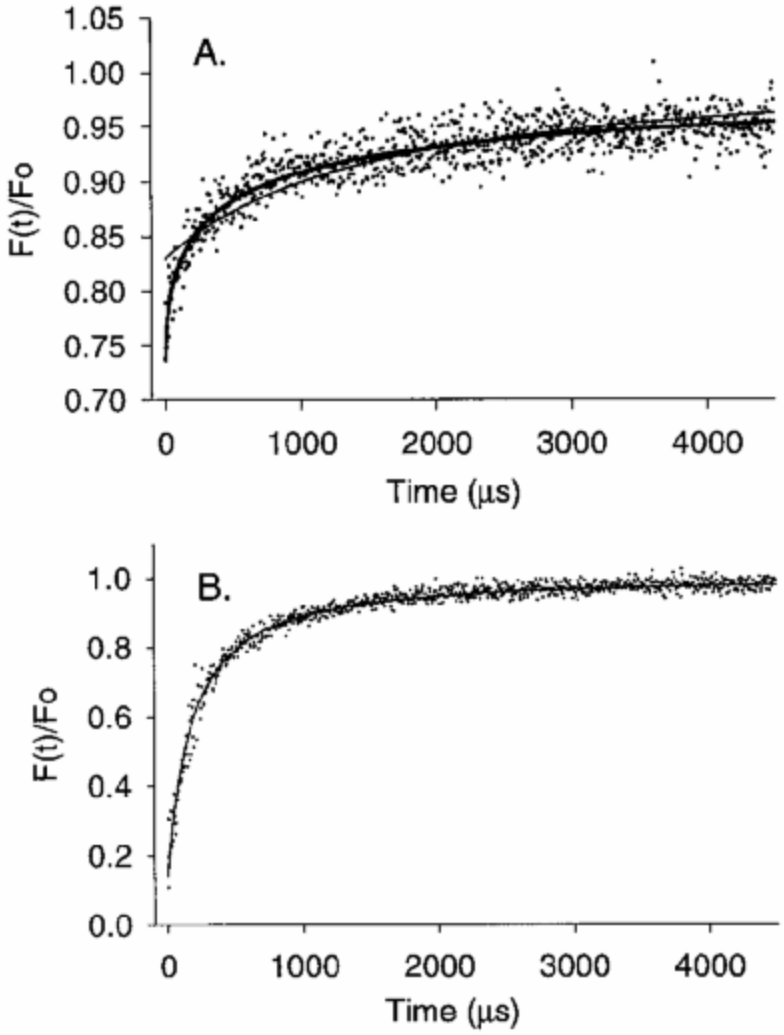
Brown et. al., 2000

Typical PAF and FRAP data



McGrath et al., 1998

Quantitative FRAP Data



Brown et. al., 2000

Fluorescence Polarizaton Decay

Basic idea: Fluorophores absorb and emit light only along certain orientation relative to the excitation light. Watch them tumble.

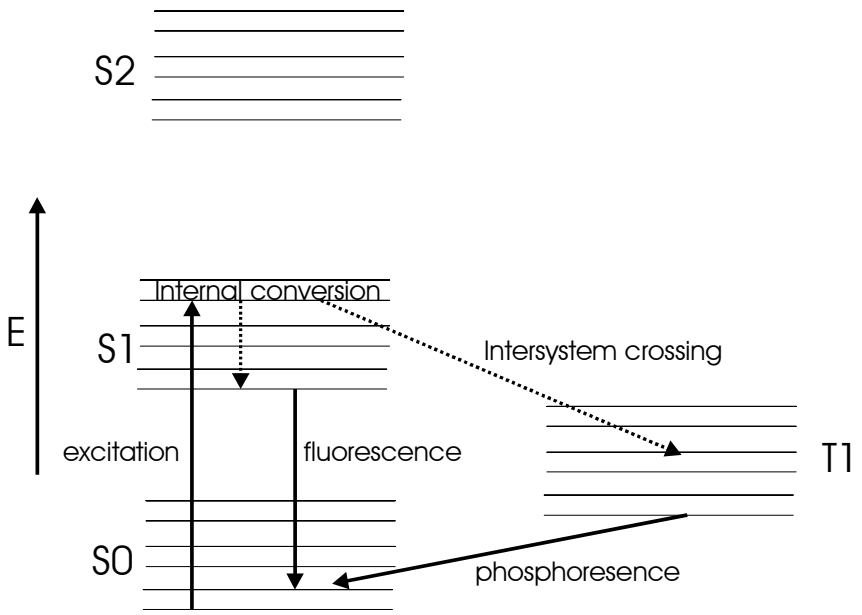
FRAP and PAF probes translational diffusion of molecules.

$$D \propto \frac{1}{r}$$

While translational diffusion is important, one should not neglect the presence of roational diffuison processes.

$$D \propto \frac{1}{V}$$

Basic Fluorescence Spectroscopy



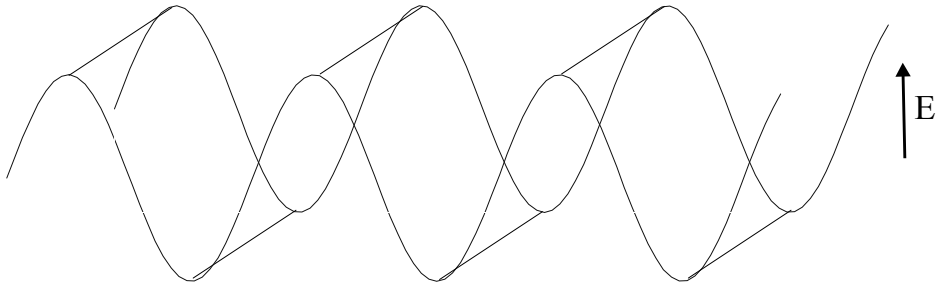
Jablonski diagram

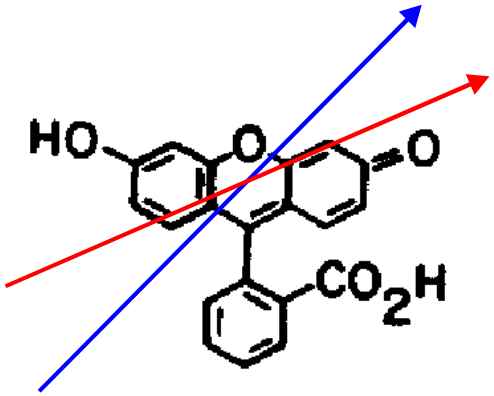
Important parameters:

(1) wavelength
(2) lifetime
(3) polarization

Fluorescence Polarization

Electromagnetic wave:

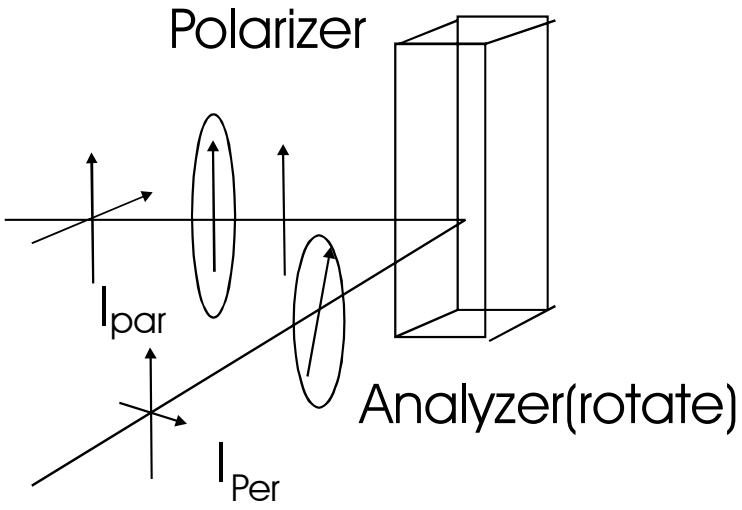




All fluorescence molecules have definite absorption and emission dipoles.

$$P \propto \cos^2 \theta$$

Rotation Measurement based on Polarization



$$P \propto \cos^2\theta$$

$$P(t) = \frac{I(t)_{par} - I(t)_{per}}{I(t)_{par} + I(t)_{per}}$$

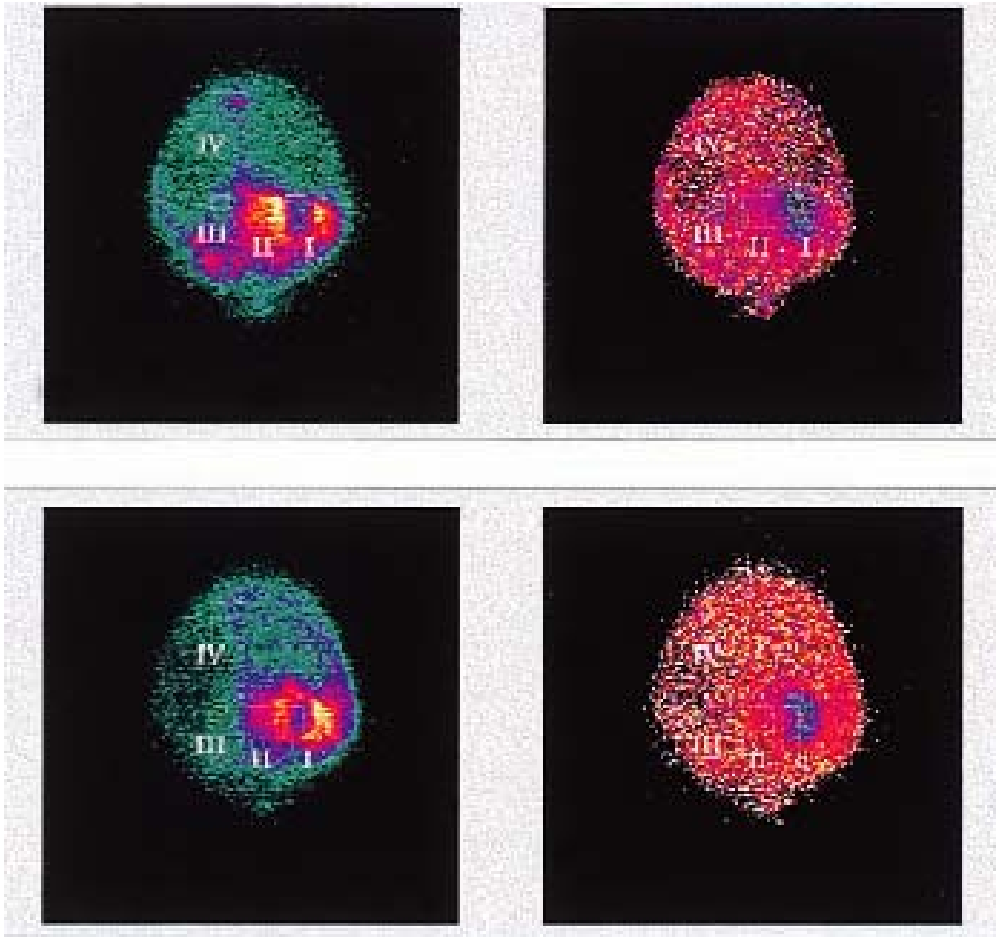
Rotational diffusion rate of molecules can be measured based on the time-resolved polarization

Typical rotational correlation time: 10-100 ps
Typical fluorescence lifetime 1-5 ns

The time-averaged polarization is zero.

Polarization need to be measured with ps time resolution.

Typical time resolved polarization images

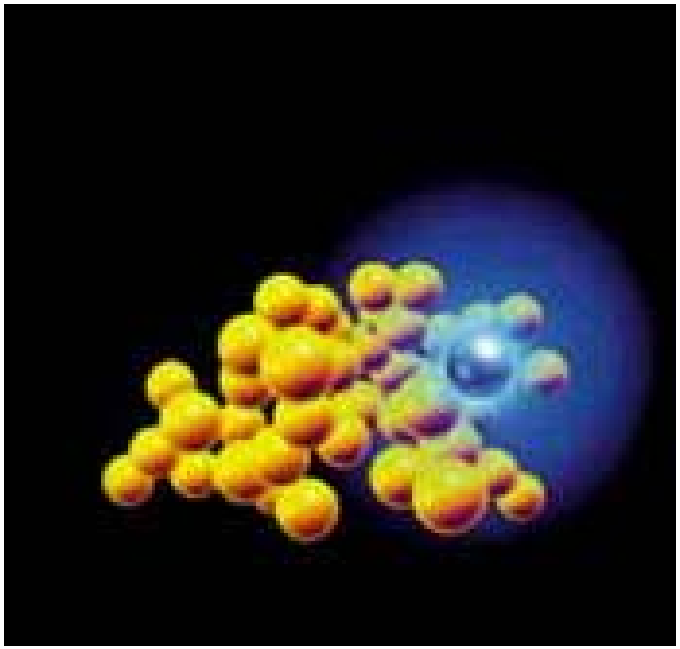


Buehler et al., 2000

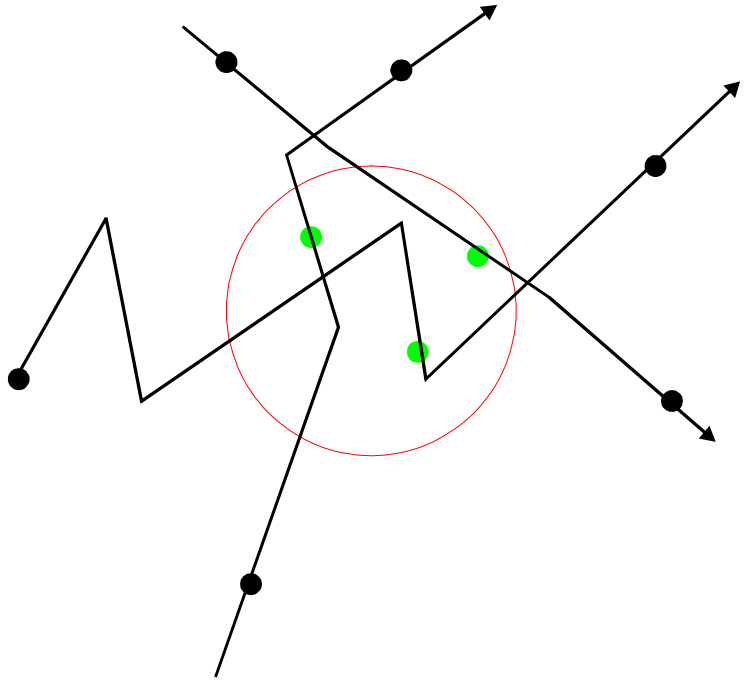
Fluorescence Correlation Spectroscopy

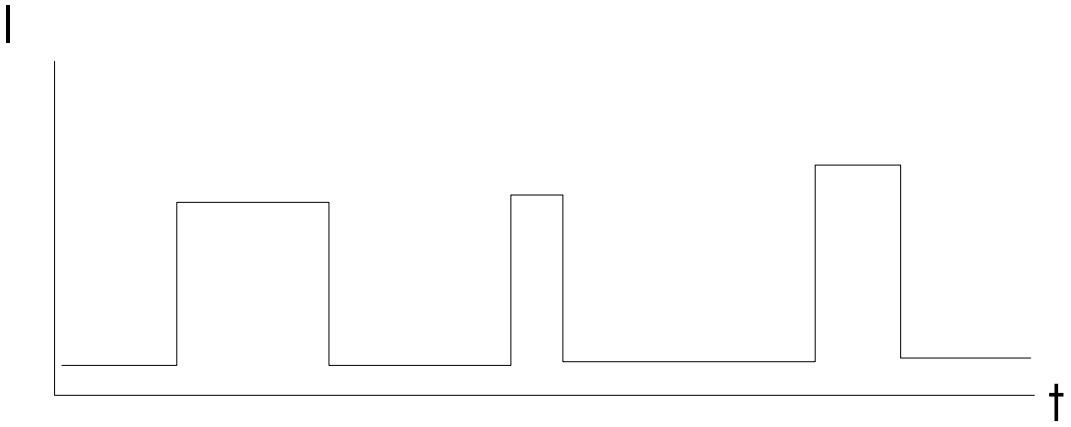
Basic idea: Look at “noise”.

If you look into a small enough volume, molecule will move in and out of it. If these molecules are tagged with a fluorophore, the detected signal with blink on and off. The temporal statistics of the blinking gives information of the molecular diffusion.

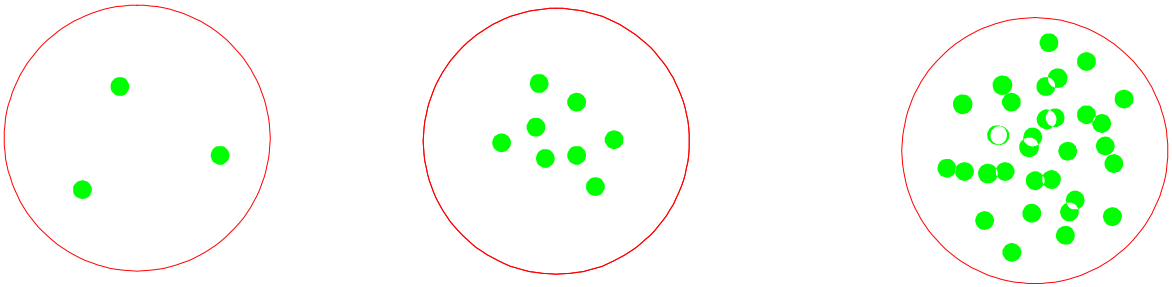


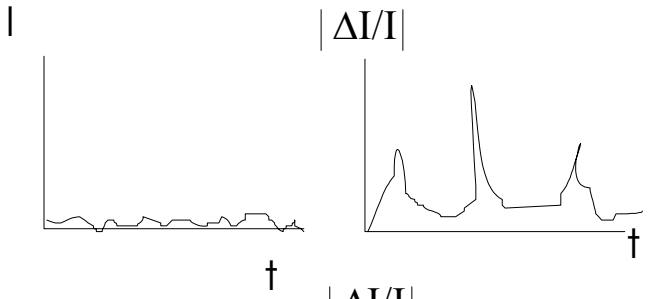
Temporal “correlation” provides
the mean transition time of the molecule
across a small excitation region.



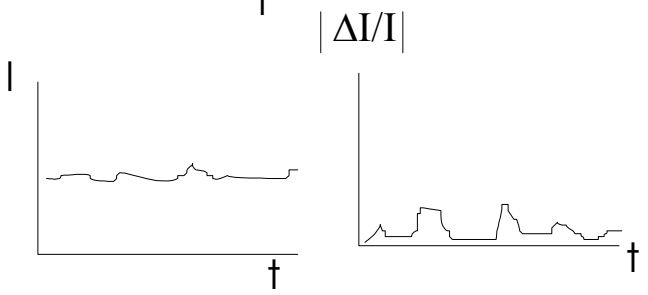


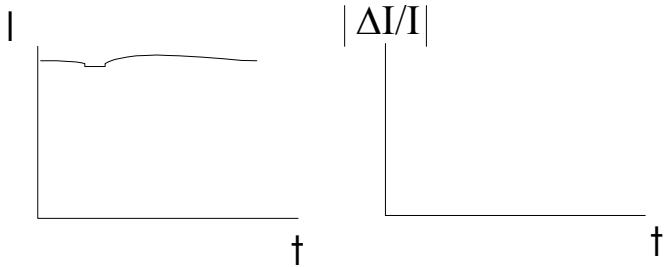
What else can we find out by looking at noise?





What does Poisson statistics tell us?



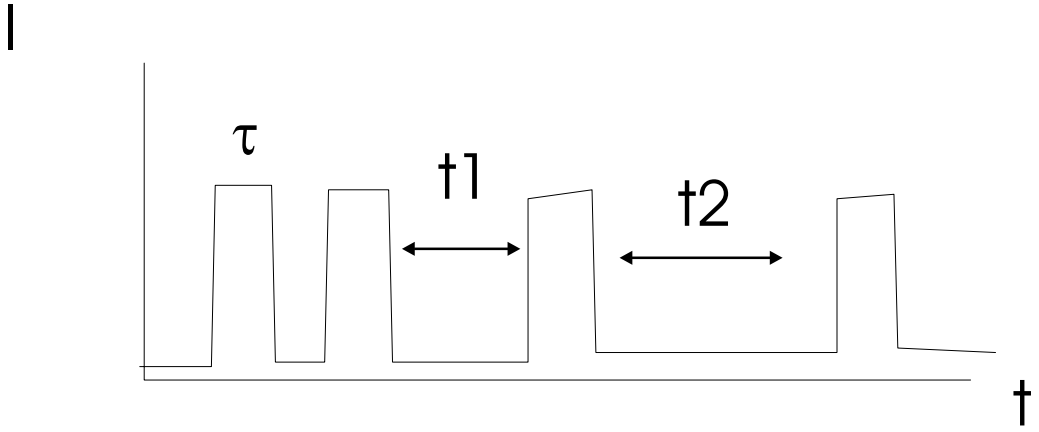


Quantification of fluctuation spectroscopy

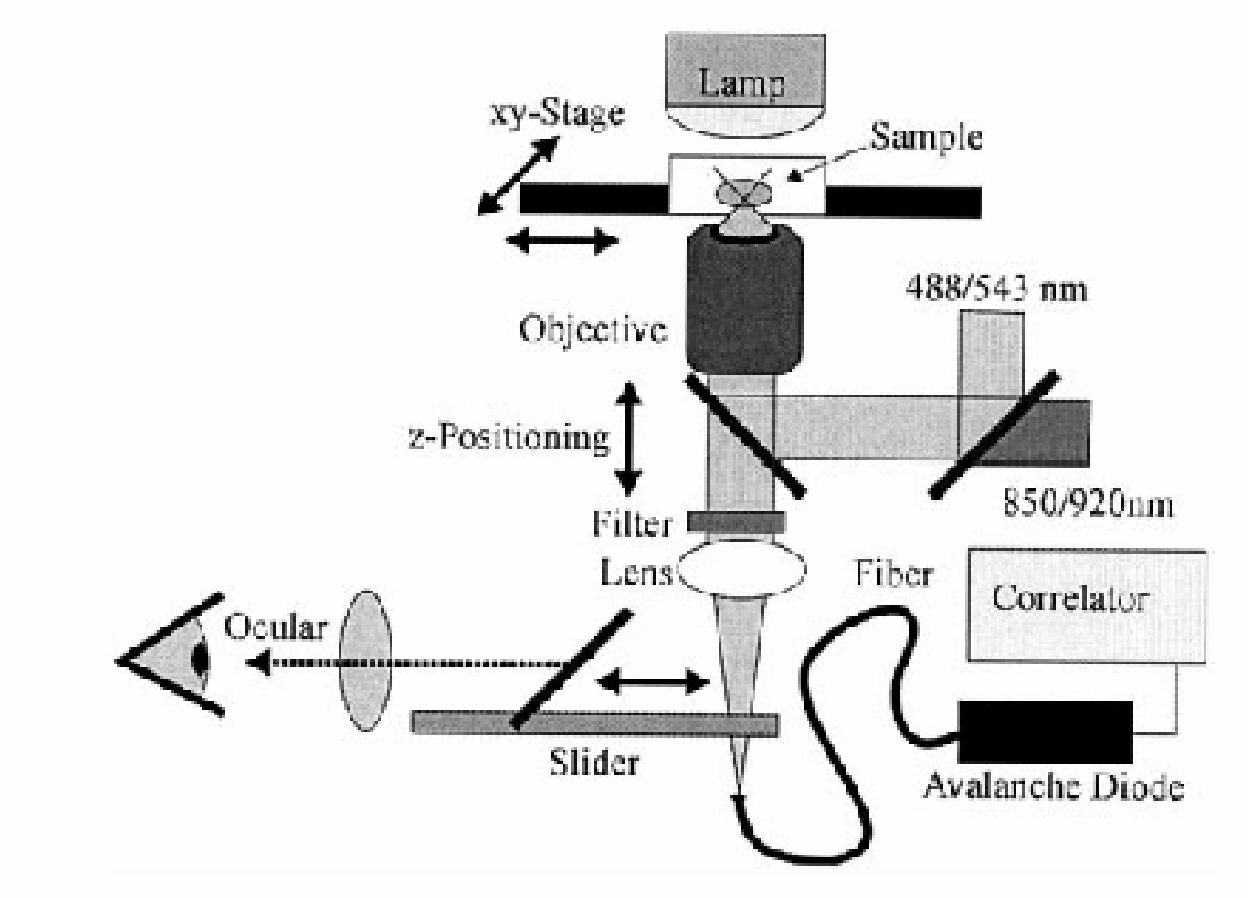
Intensity fluctuation is typically analyzed using the autocorrelation function:

$$g(\tau) = \frac{< I(t)I(t+\tau) > - < I(t) >^2}{< I(t)^2 >}$$

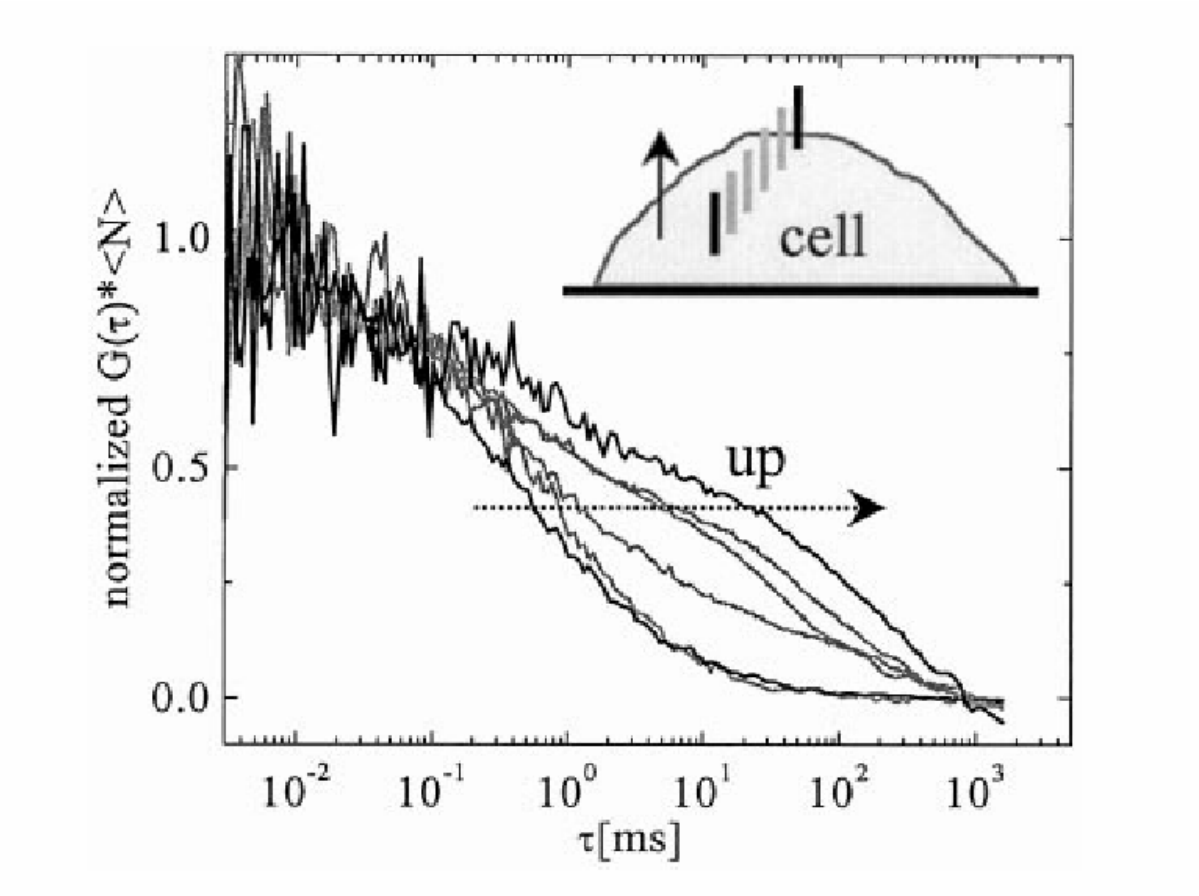
What does it mean? It is a measure of this: if you are measuring a high intensity at a given moment, what is the chance that you will still measure a high intensity some time τ away.



Instrumentation for FCS



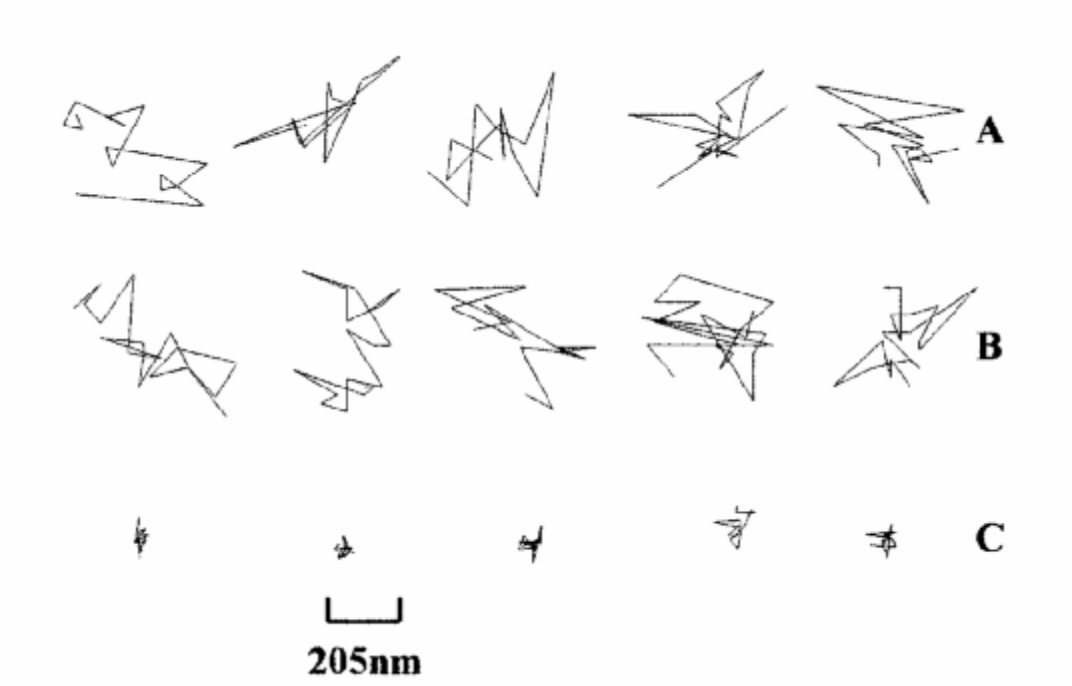
Monitoring Diffusion of GFP-lipid in Cells



Schwille et al, 2000

Single Particle Tracking

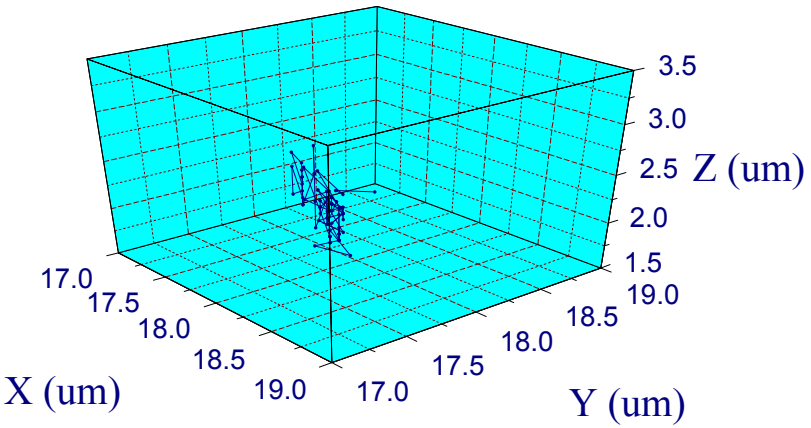
Basic idea: Ensemble average does not tell the whole story. Watch the diffusion/transport of single molecular motion in cells. Very useful for discern non-stochastic modes



Smith et al, 1999

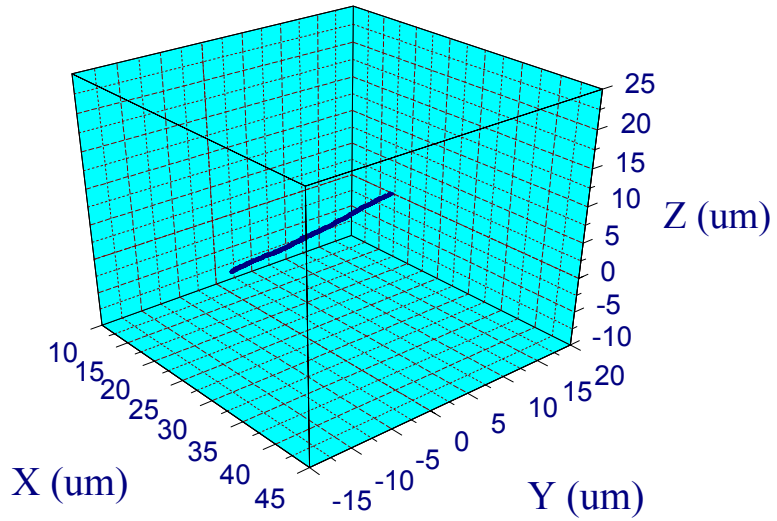
Typical tracking data of diffusion particles under thermal current

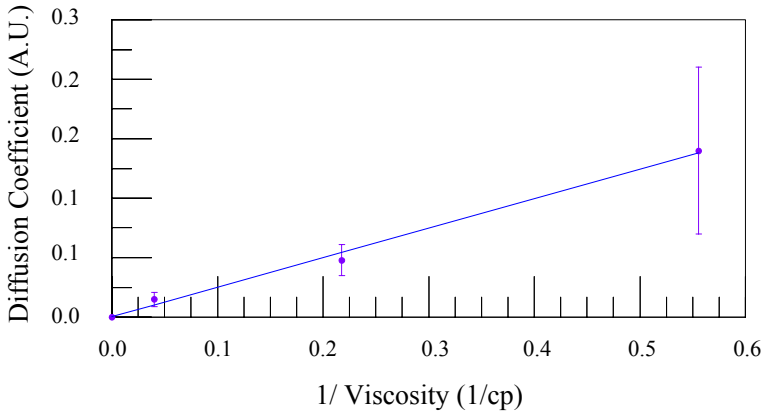
Short Term Tracking



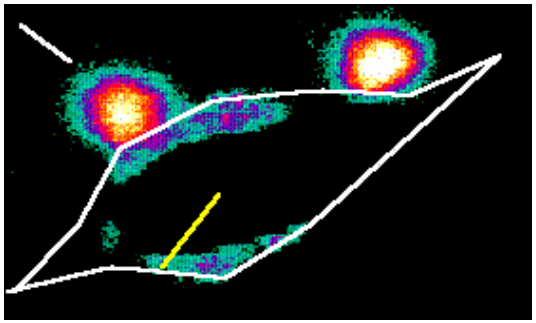
The details in the trajectory allows us to separate out diffusive from driven motion.

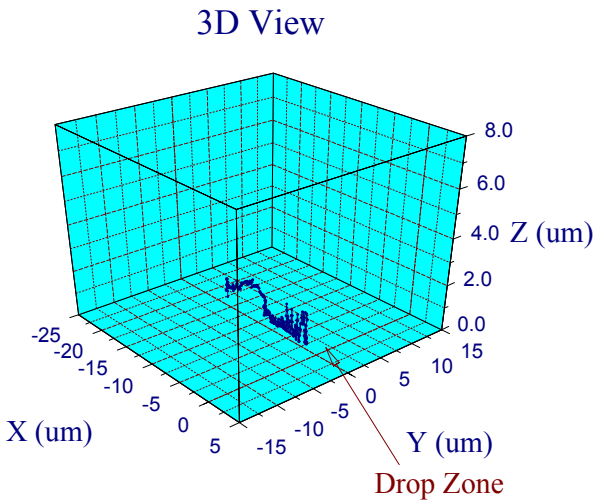
Long Term Tracking

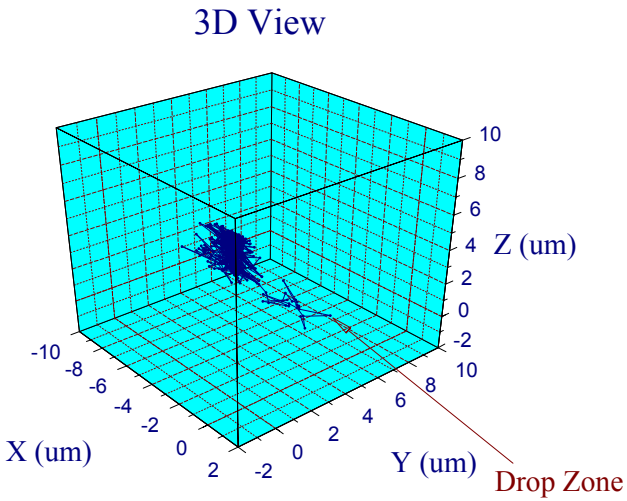


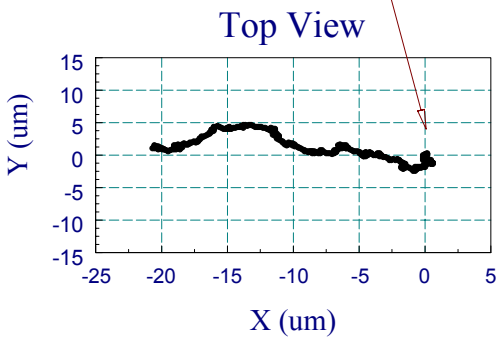


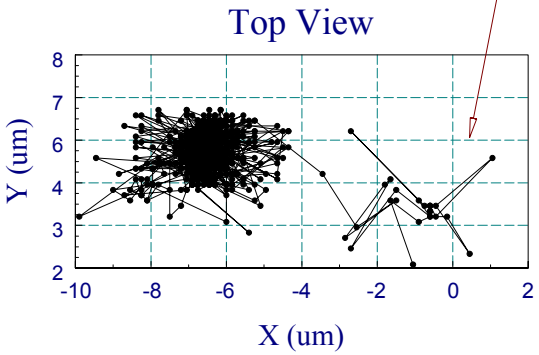
Macrophage capture of BSA coated particles







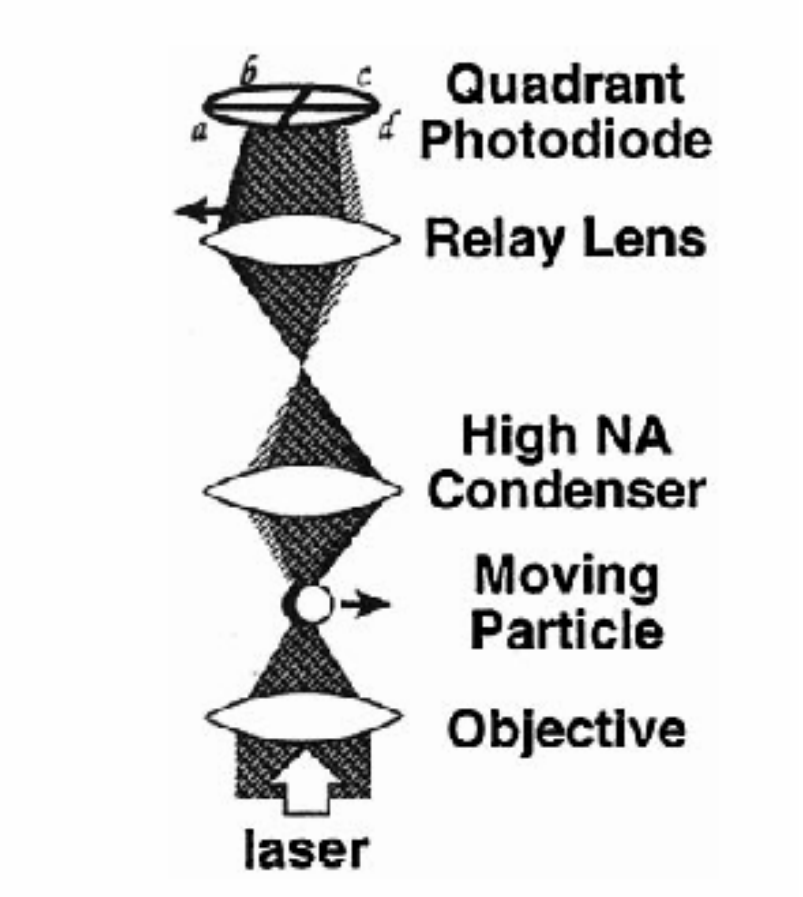




Laser Tracking Microrheology

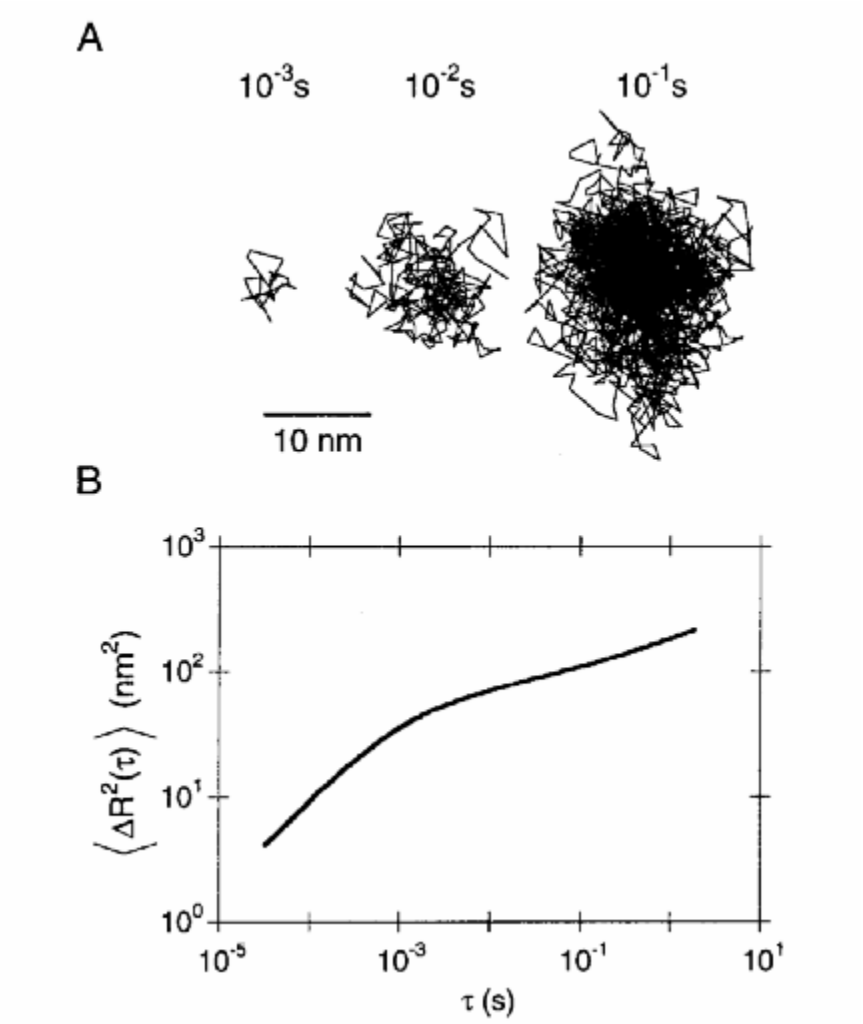
Basic idea:

Extending SPT to a much faster time scale.
Instead of imaging the motion of particles over a whole image ,
LTM focus on a single particle tracks it with excellent spatial and time resolution.



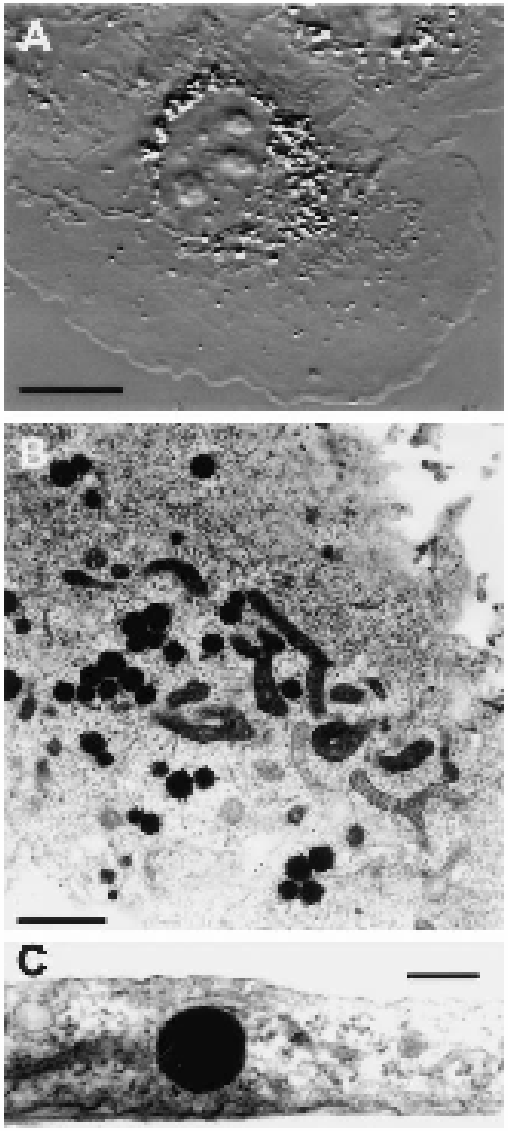
Yamada et al., 2000

Typical Tracking data of LTM



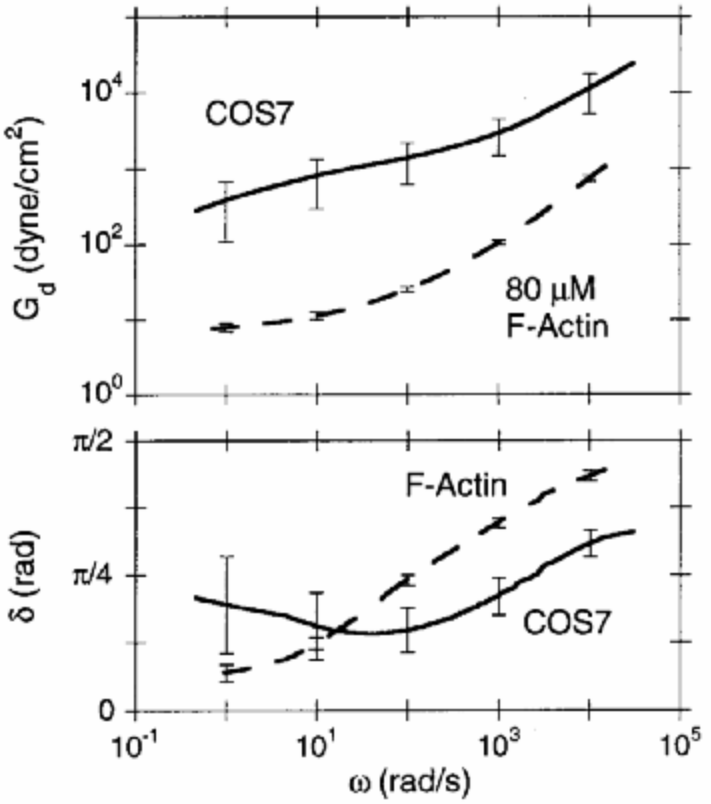
Yamada et al., 2000

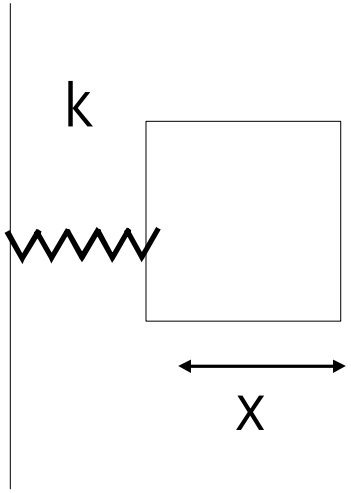
Tracking granules in kidney epithelial cells



Yamada et al., 2000

Rheology data gained from tracking data of intracellular granules





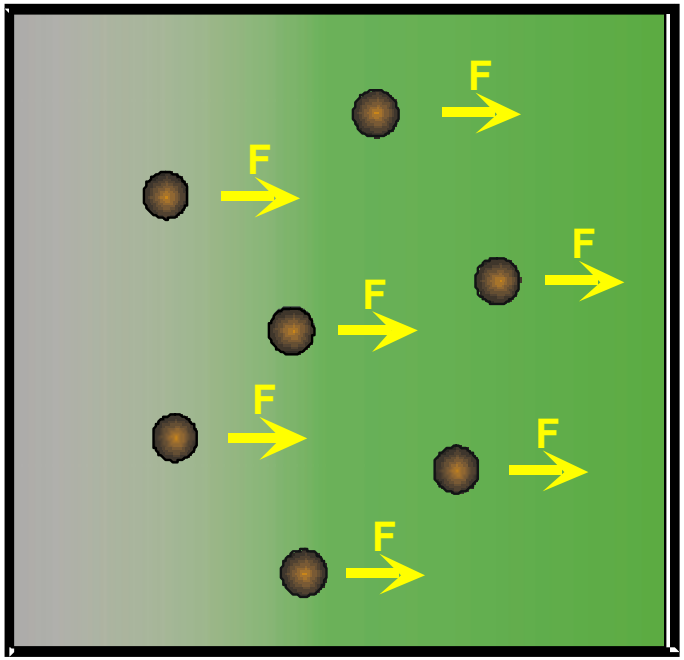
Equipartition theorem:

$$\frac{1}{2}k < x^2 >= \frac{kT}{2}$$

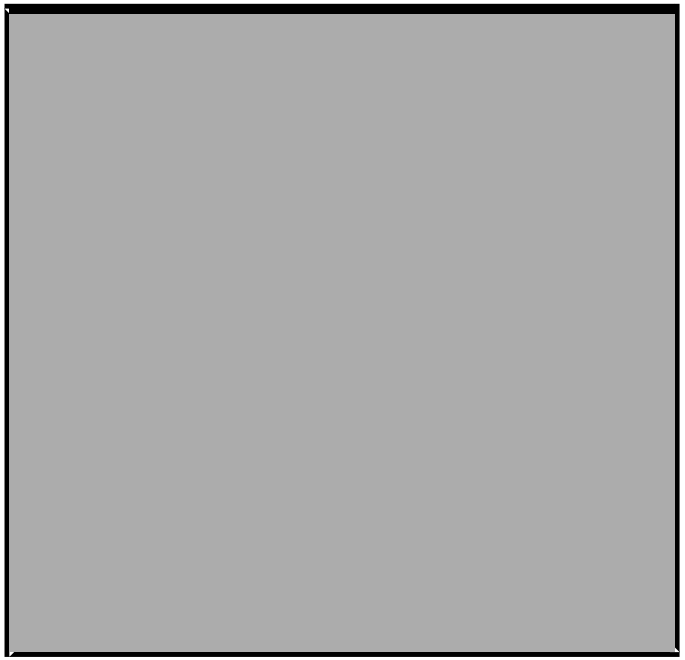
Yamada et al., 2000

Magnetic Versus Optical Manipulation

Magnetic: constant force

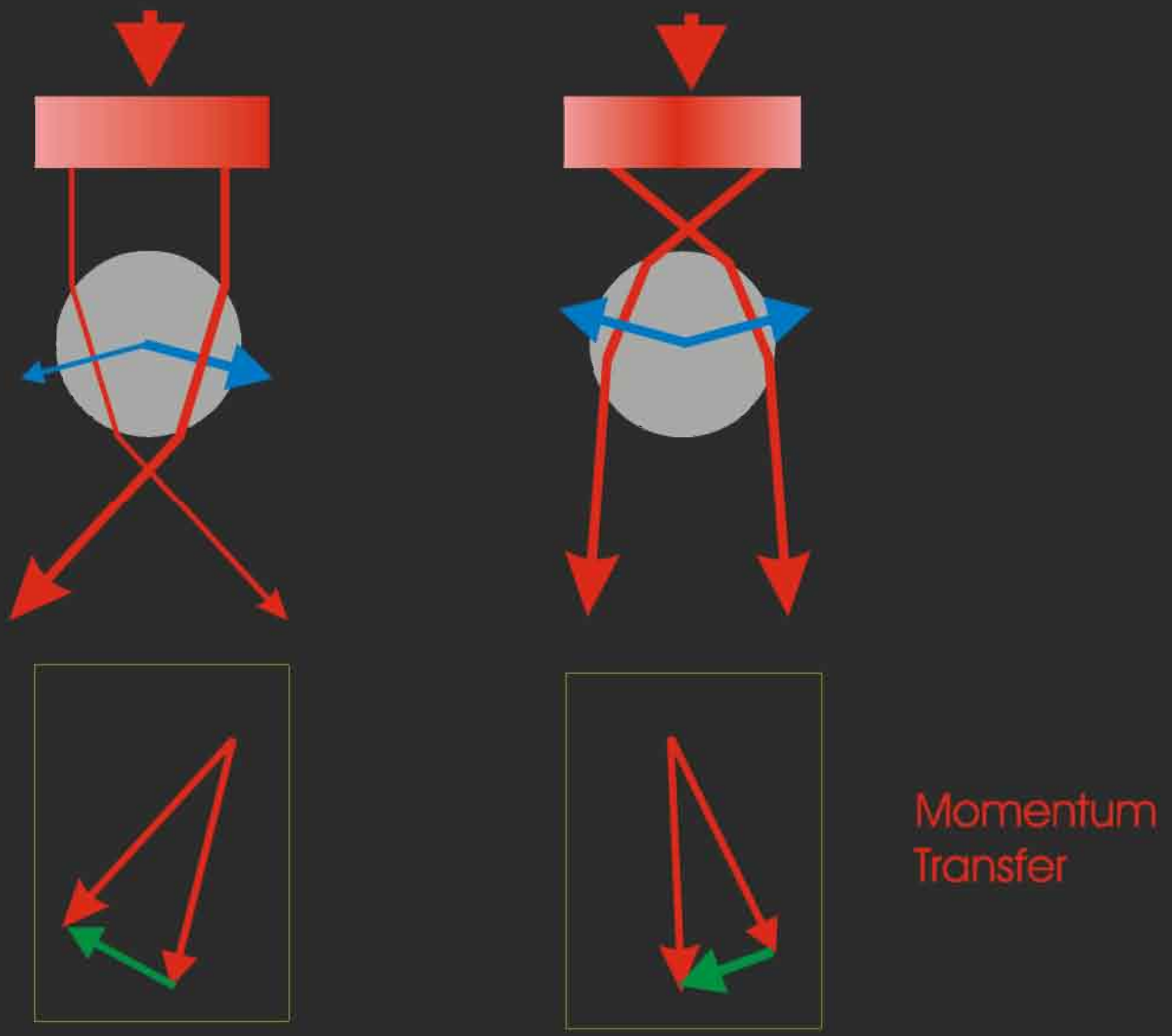


Optical: constant position



Magnetic	*Optical*
Uniform force across sample	Force level depends on location within trap
Force set by magnet current	Requires particle location measurement to determine force
Large force range: 0.01 - 500 pN	Force range limited by Low: Brownian fluctuations for force determination High: Sample damage by high laser intensities
Rotational fields generate torque	Difficult to implement rotational manipulation
Simultaneous parallel manipulation	More difficult for multiple manipulation

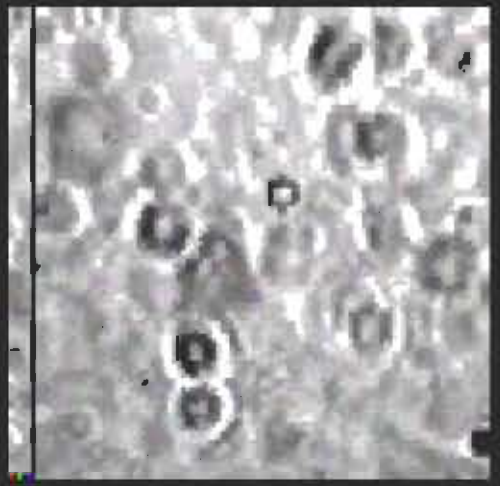
Basic Principle of Laser Tweezers



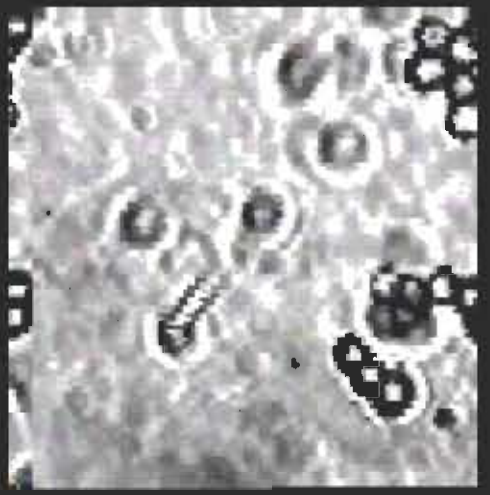
Svoboda&Block, Ann. Rev. Biophys. Biomol. Struct., 1994

Some exercises with optical tweezers

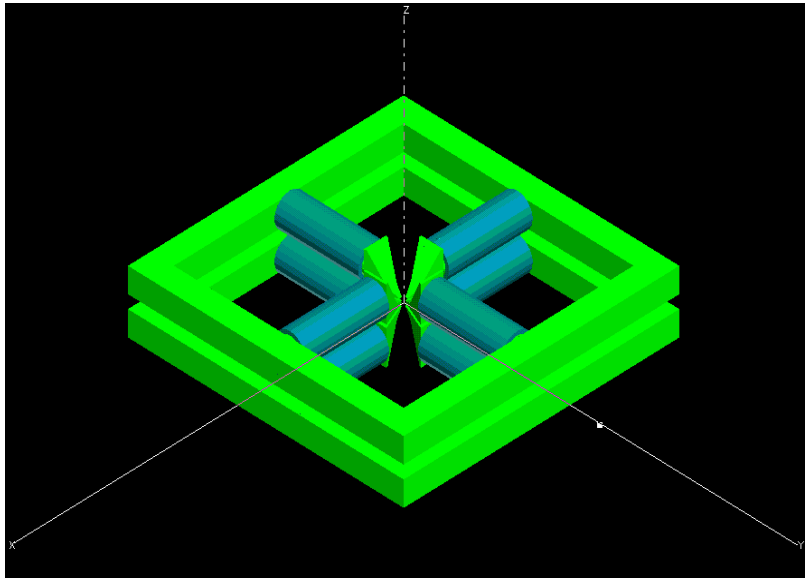
A single bead



DNA linked beads

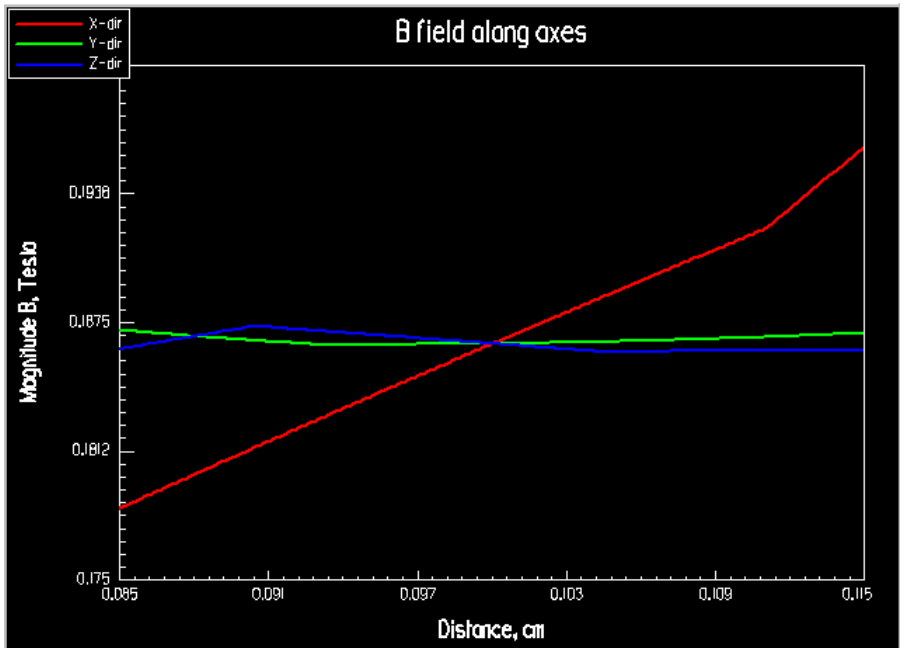


Magnetic Trap



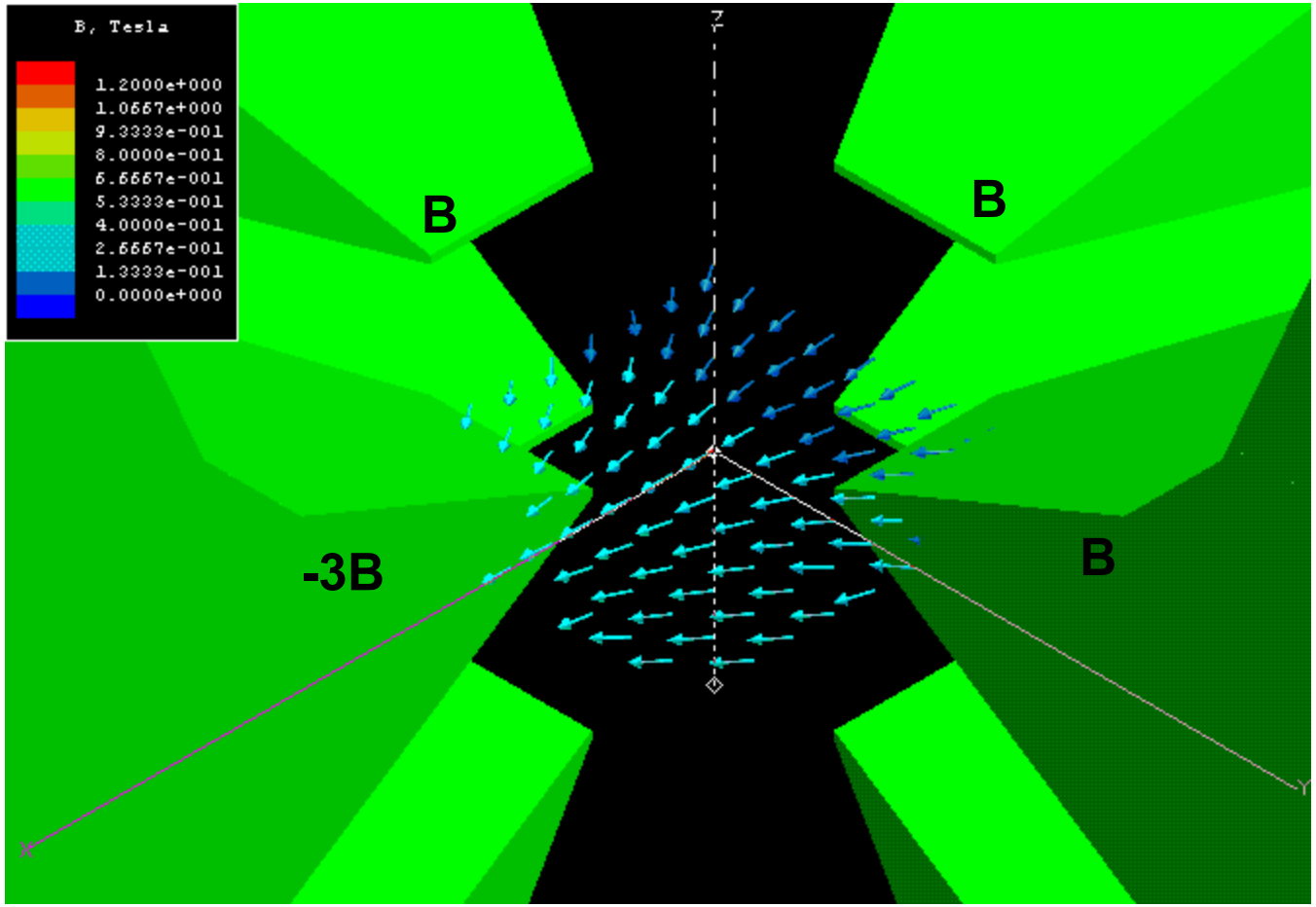
The geometry of the magnetic trap is shown at the left. The sample is placed in the center.

The force is generated by a combination of the field strength and field gradient. It is possible to obtain 250 pN of force per bead.



Initial design: Amblard et al., RSI, 1996

Force Generation By The Magnetic Manipulator



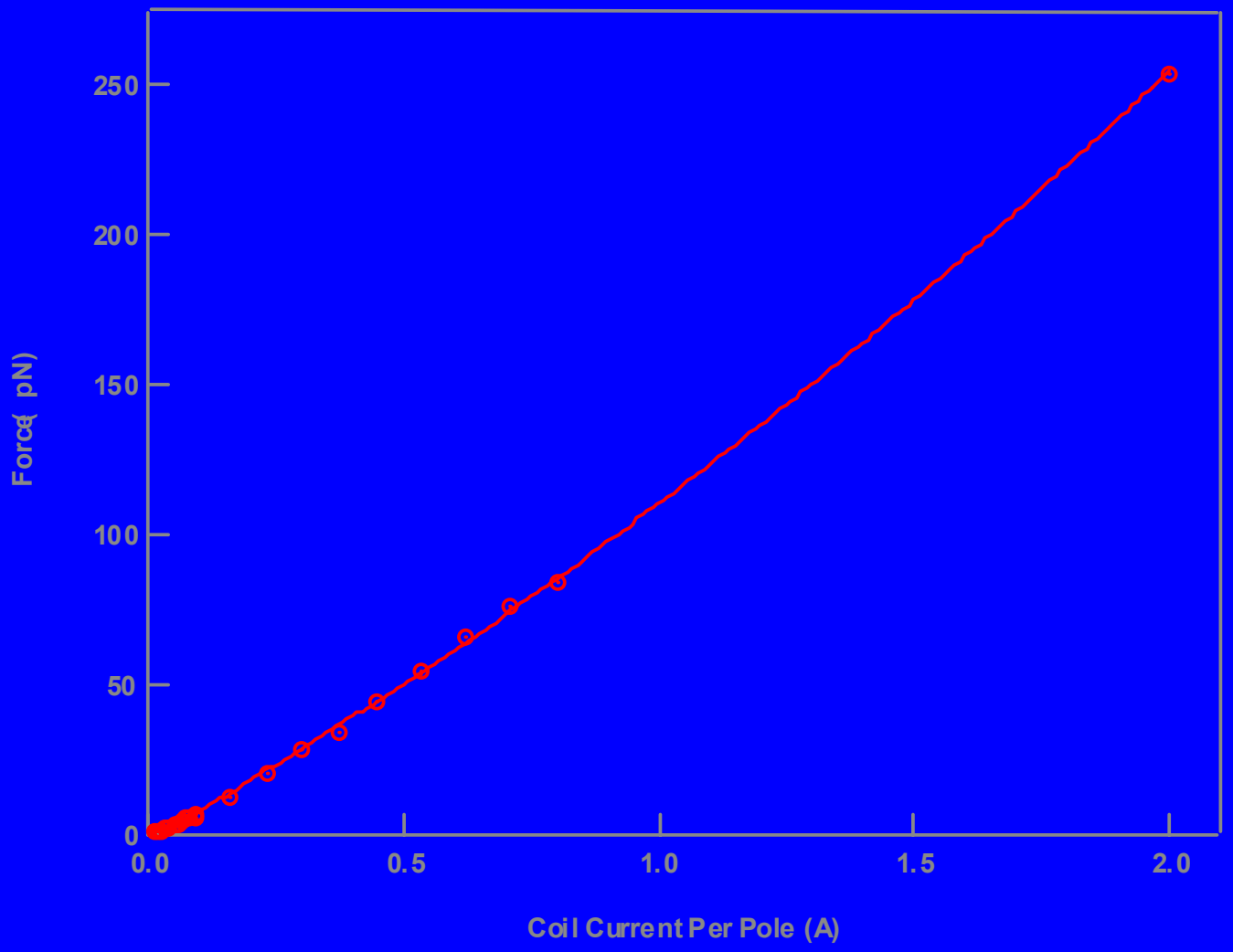
$$\vec{F} = (\vec{m} \bullet \nabla)\vec{B}$$

$$\vec{m} = \chi V \vec{B}$$

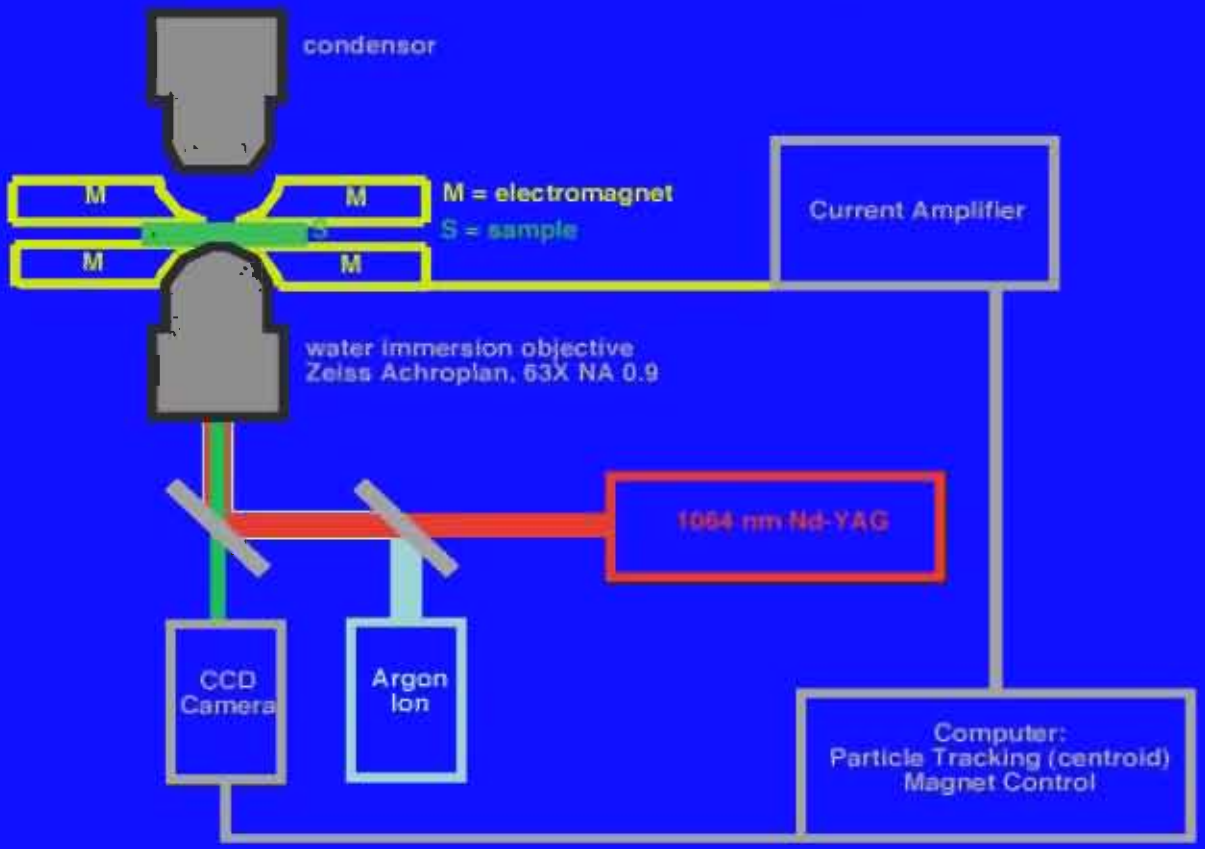
$$= \text{induced bead magnetic moment}$$

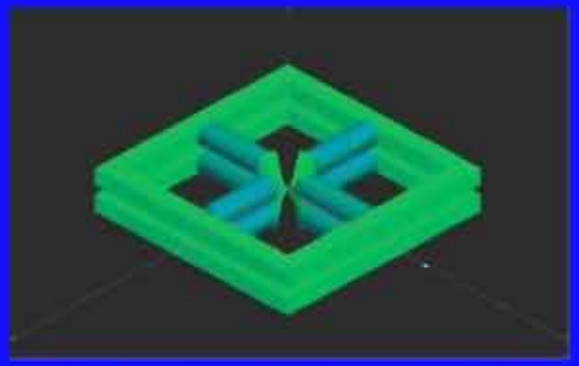
Force depends on both magnetic field strength and gradient

Magnet Force Calibration



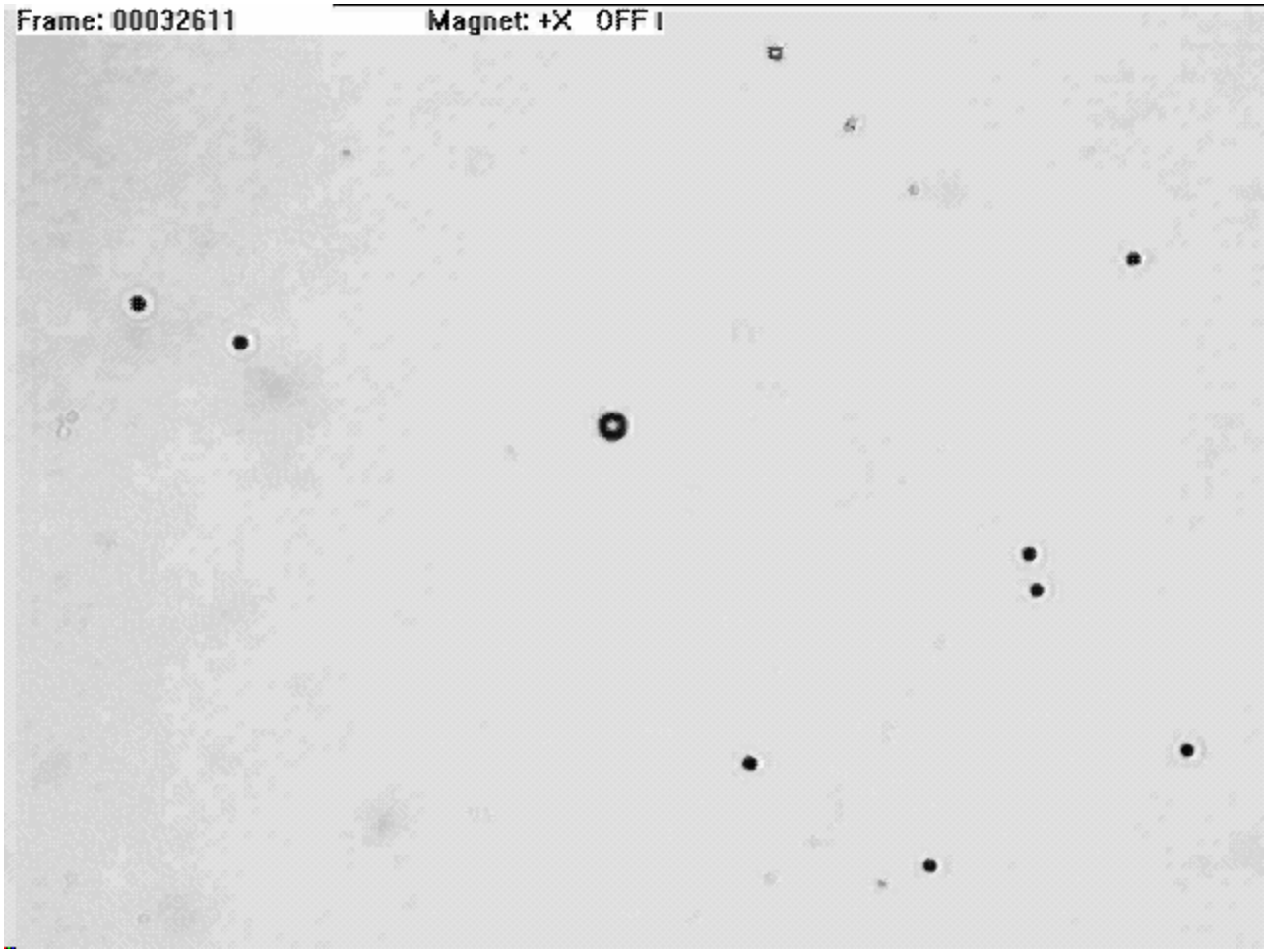
Microscope Schematic



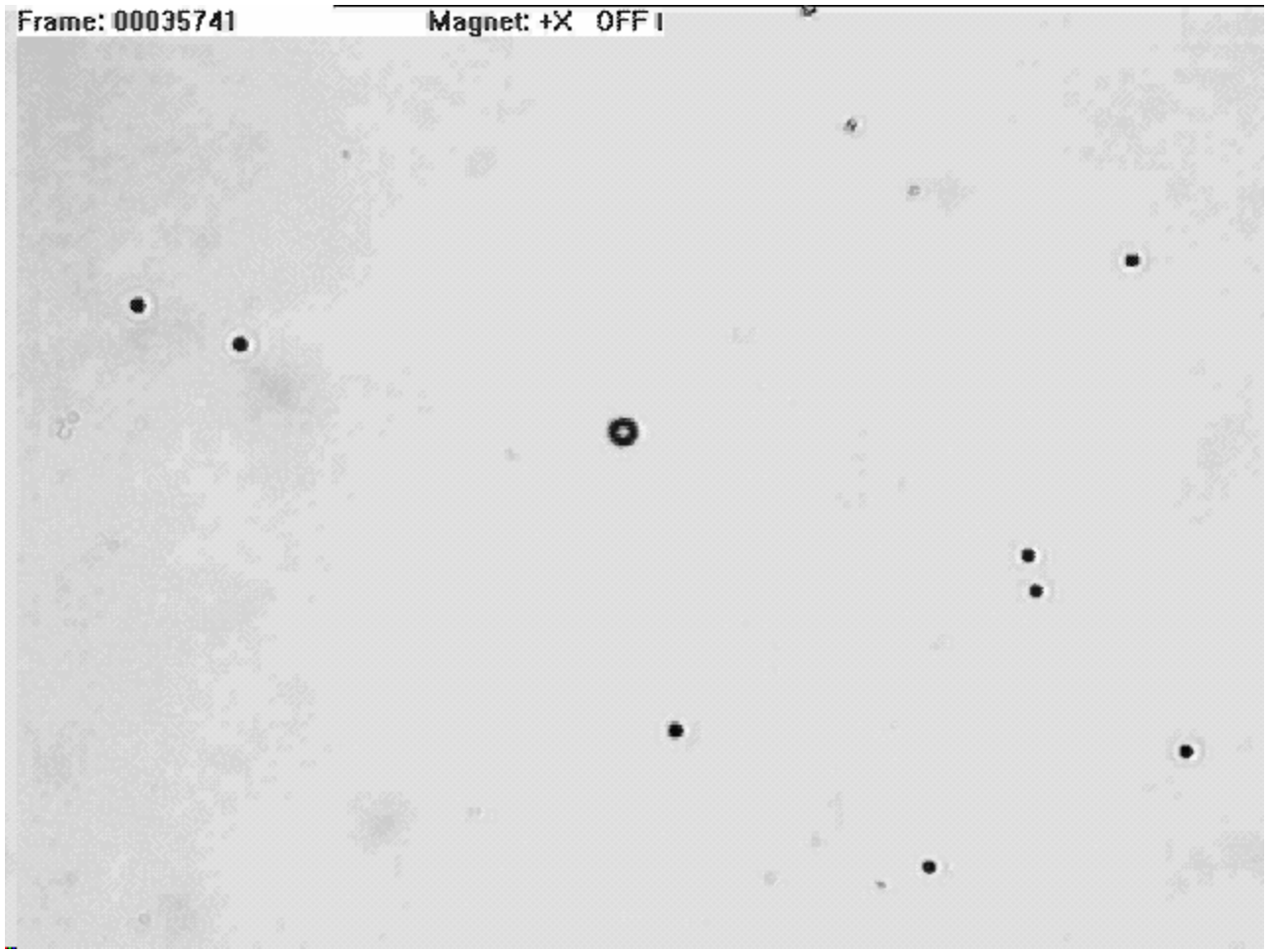


Perspective View of 8 Pole Magnetic Manipulator

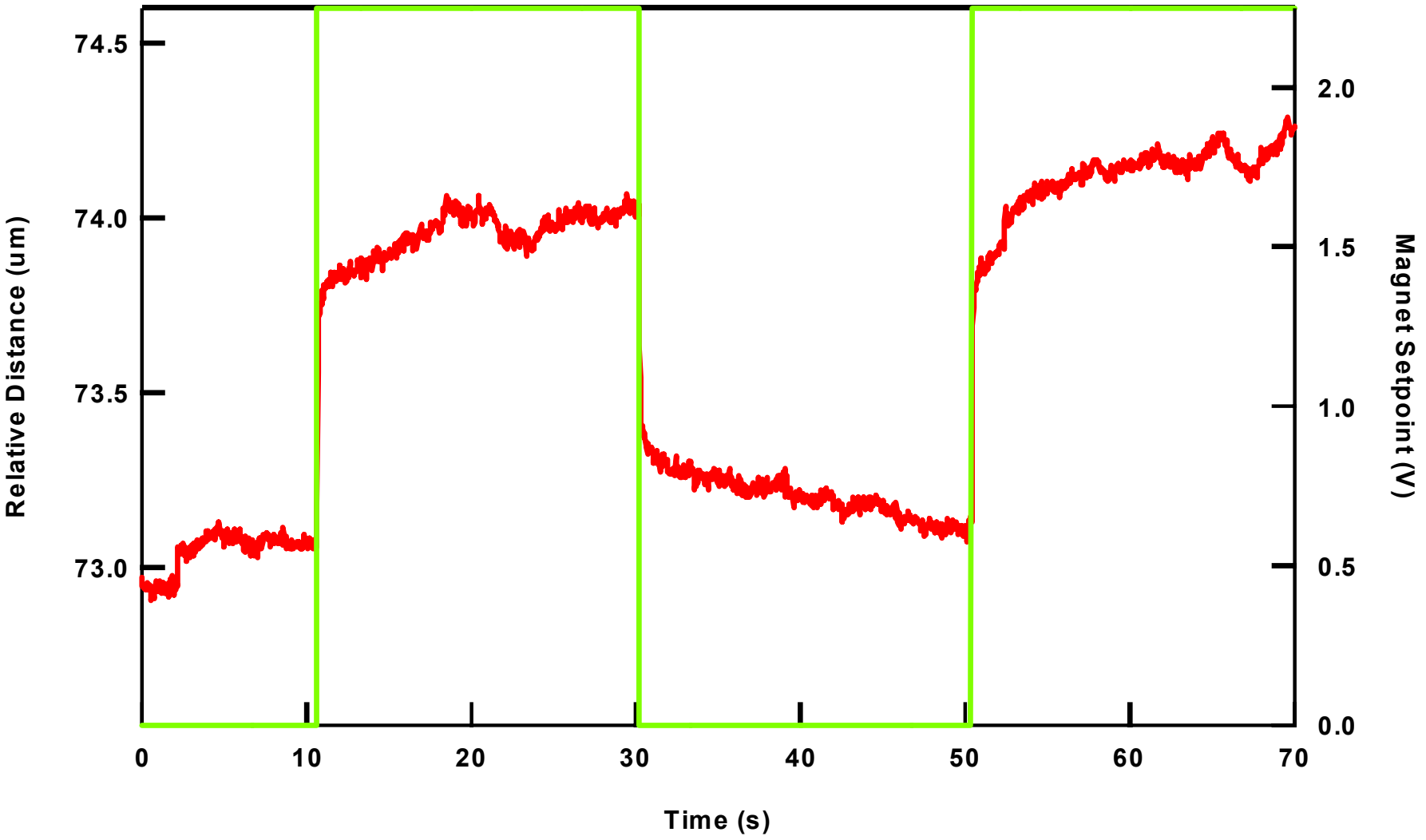
Magnetic manipulation of DNA-EXO Complex



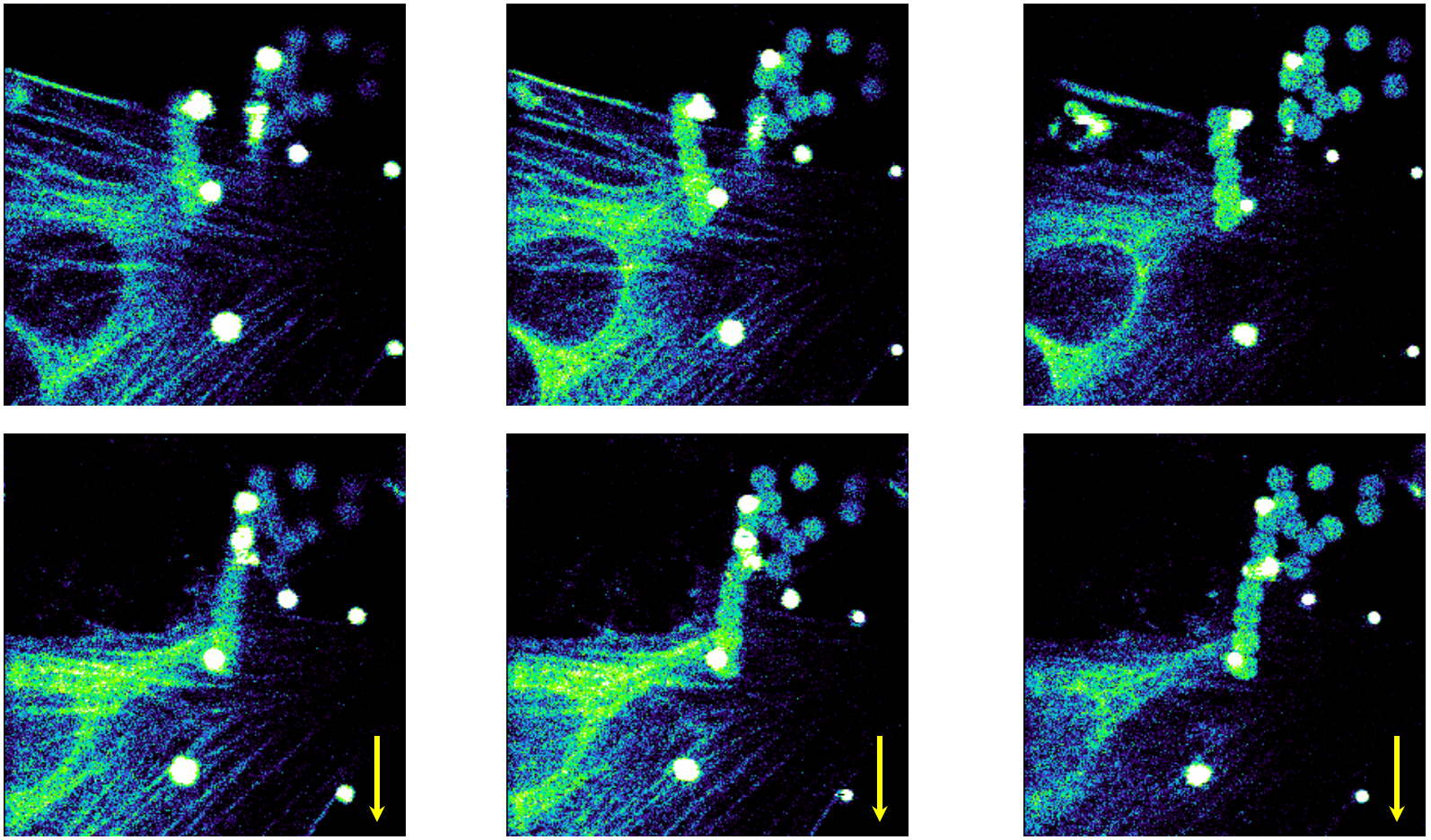
Wilder Manipulation of DNA-EXO Complex



CELLULAR RESPONSE TO MAGNETIC FORCE

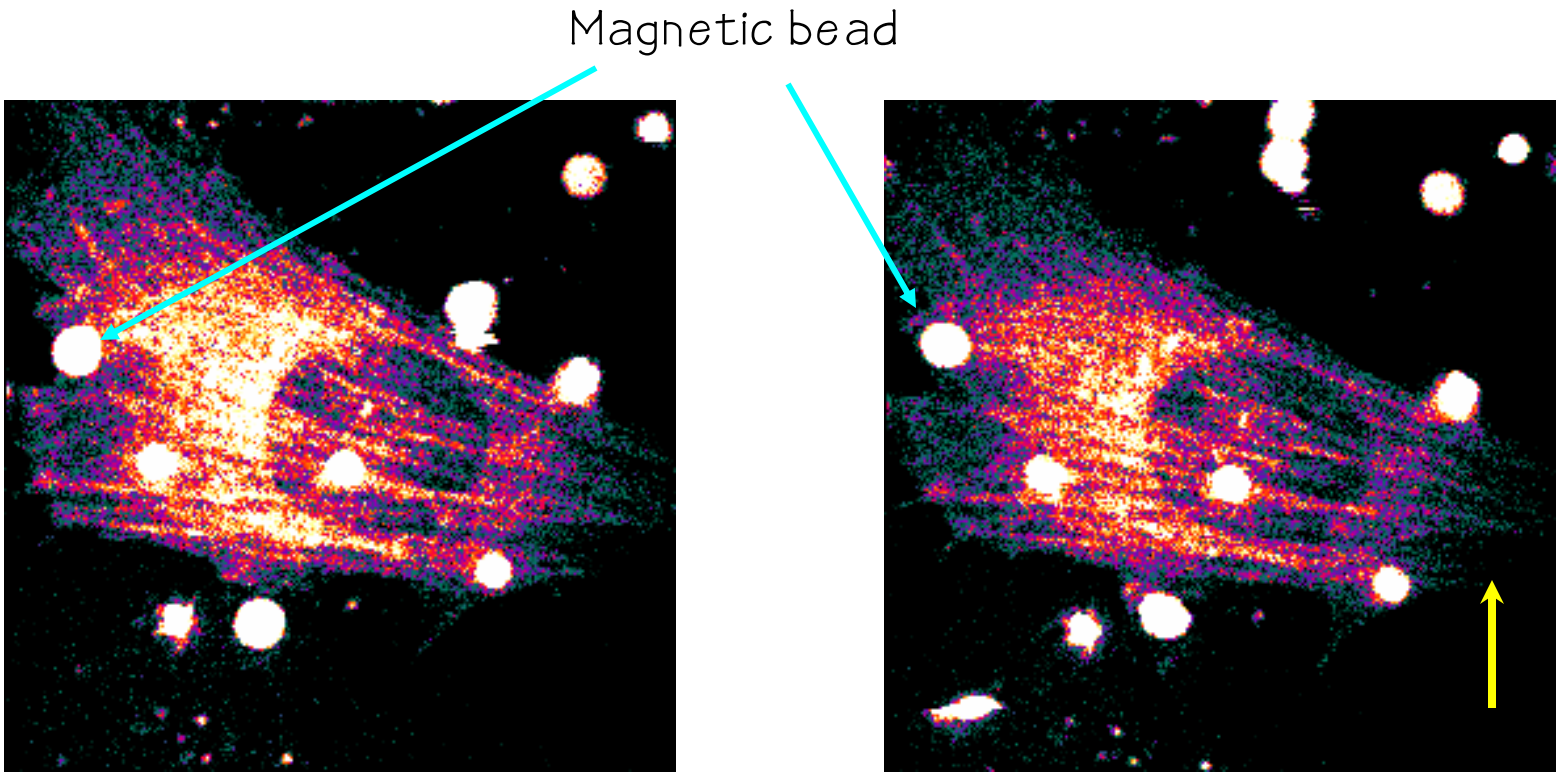


3-D Distribution of GFP-actin



Same cell, top three with no force, bottom three with a 200 pN force in the arrows' direction. Slices are 250 μm apart. The lowest slice is on the left.

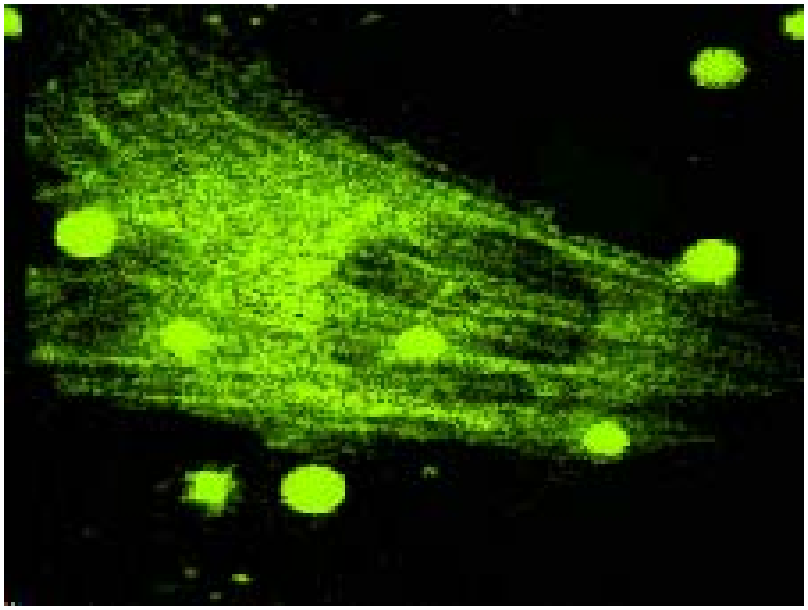
Force Applied, 2-D section



Before a force is applied.
All beads are polystyrene
except as indicated

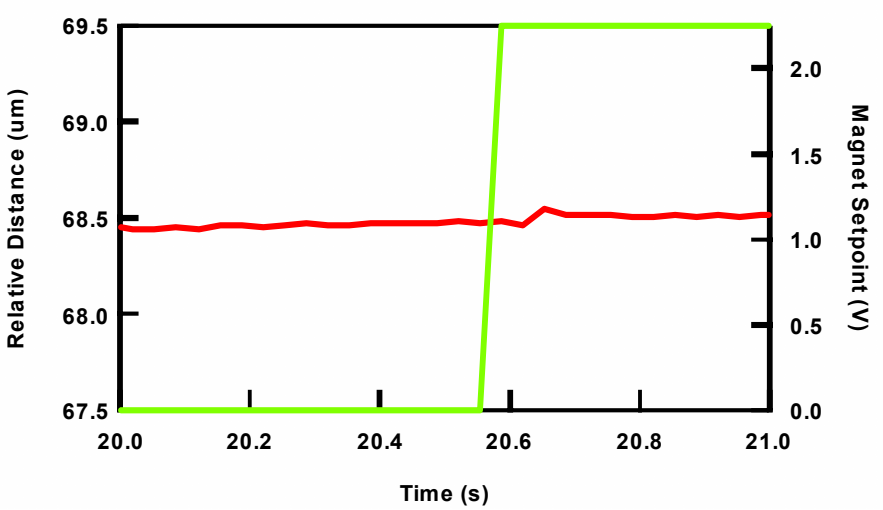
After a 200 pN force is applied
in the direction of the arrow.

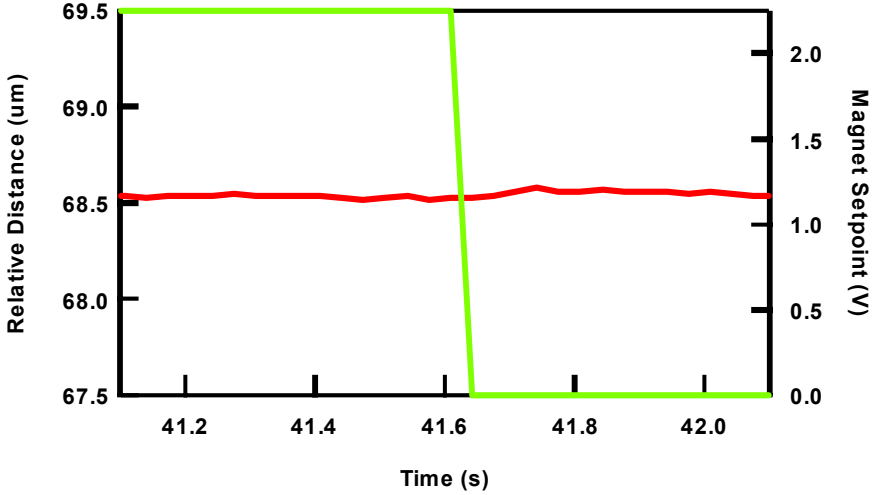
Non-Local Cytoskeletal Deformation From Localized Strain

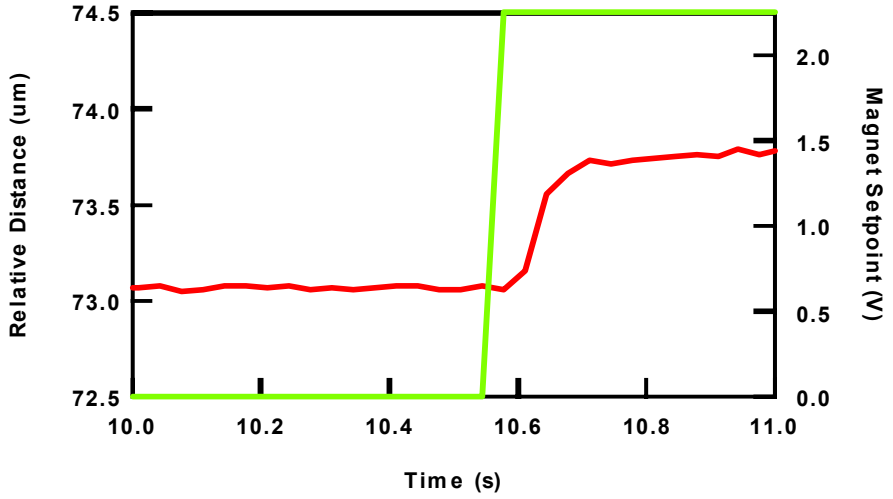


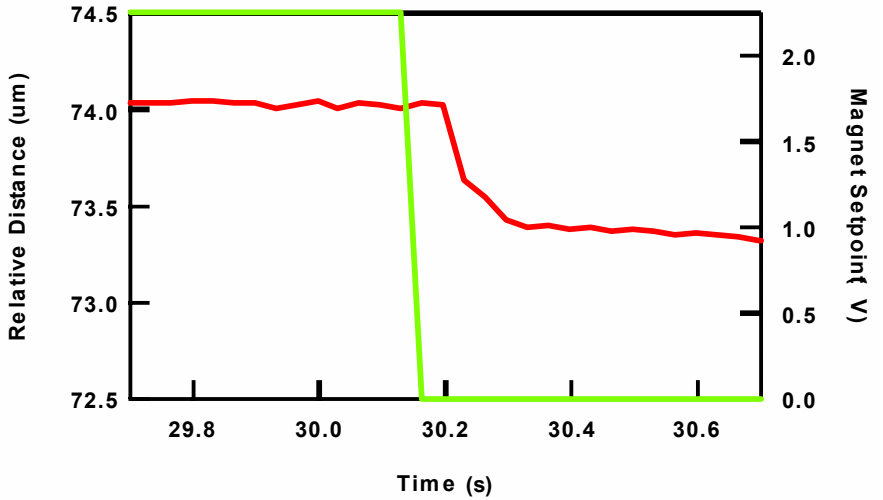
GFP-Actin transfected human arotic smooth muscle cells. Super-paramagnetic particles are attached to cytoskeleton via fibronectin-integrin linkage. 100 pN per bead is applied using a magnetic micromanipulator.

INDUCED MOTION
(top: control, bottom: cell)









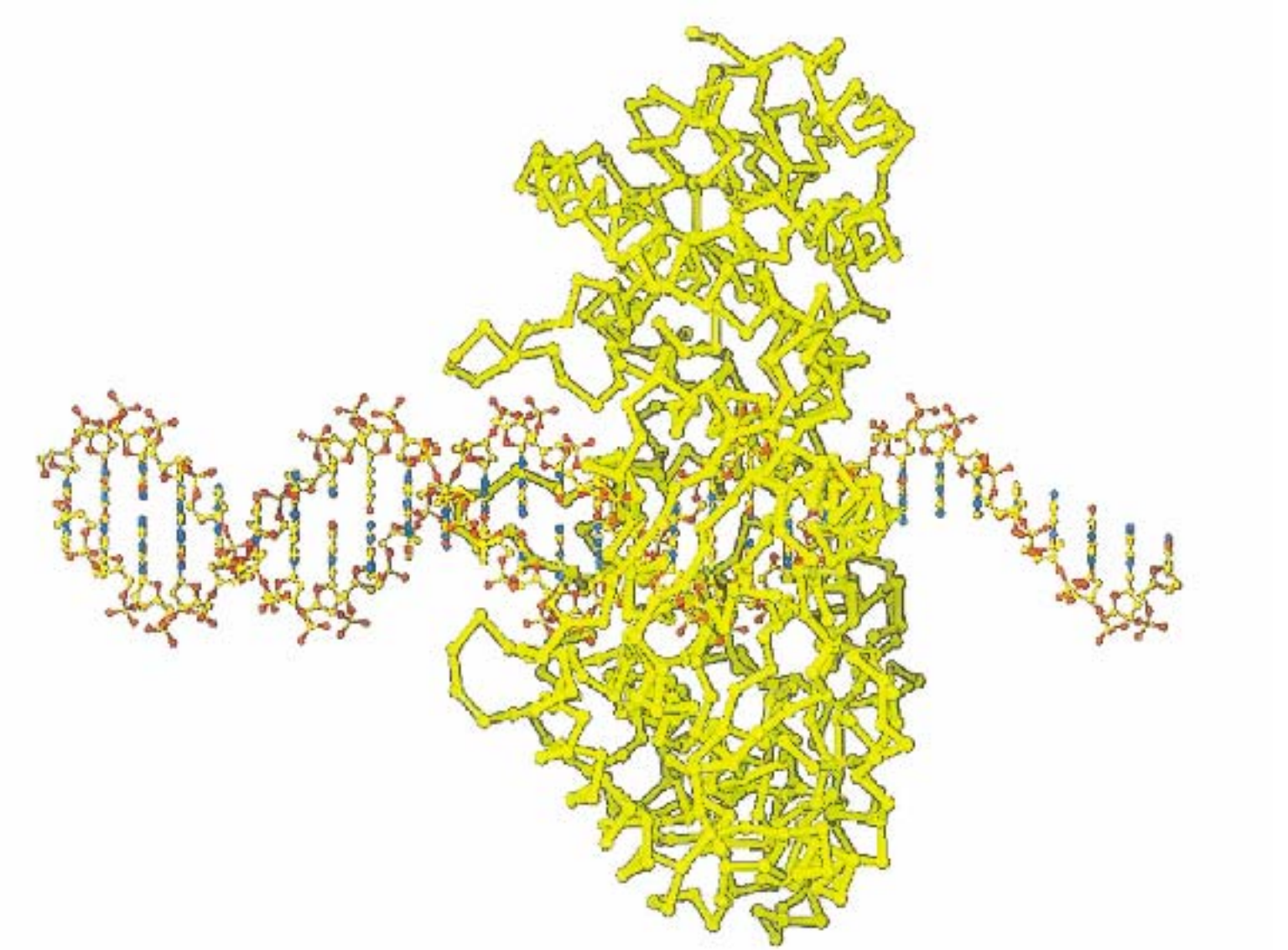
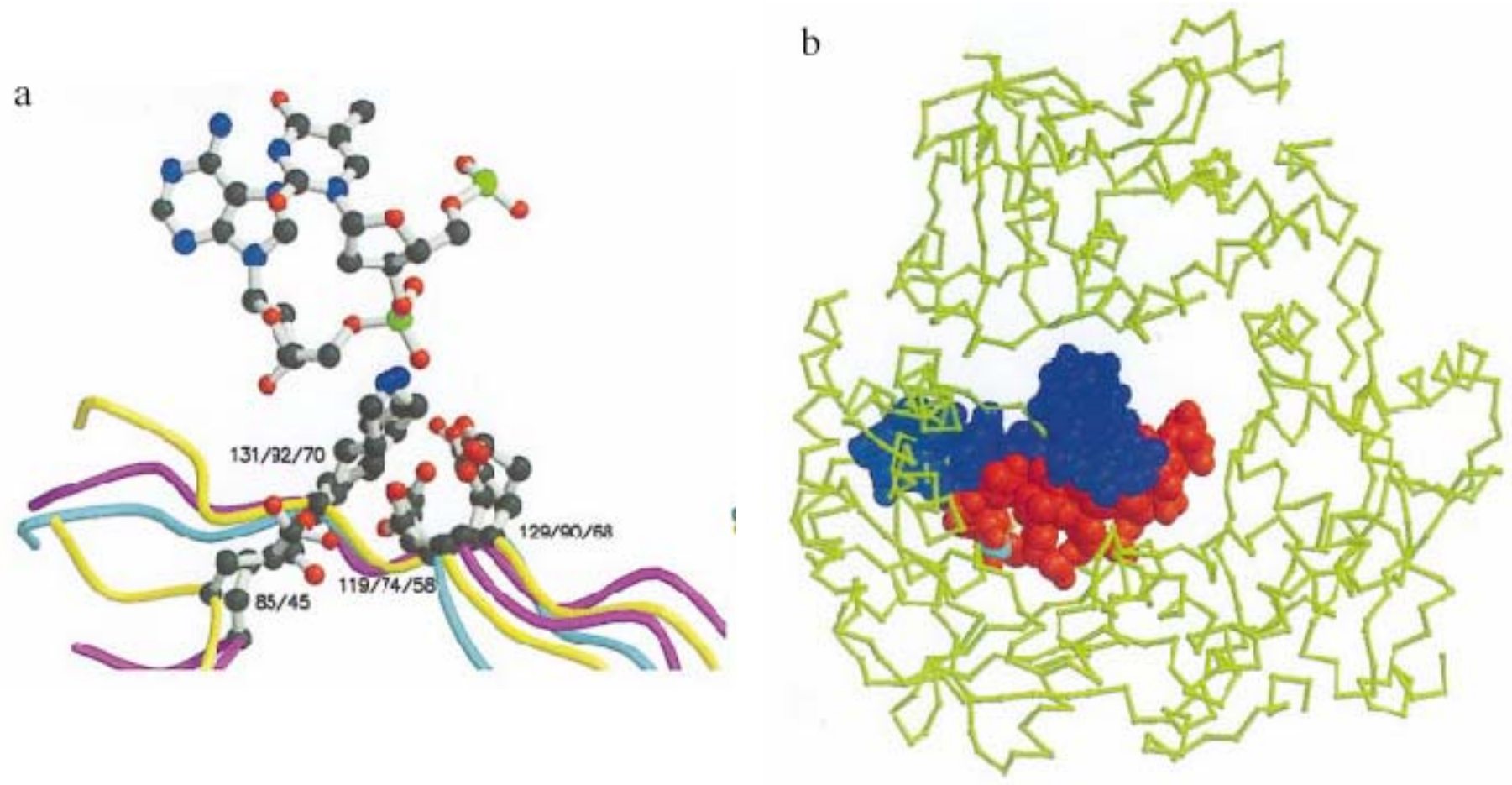
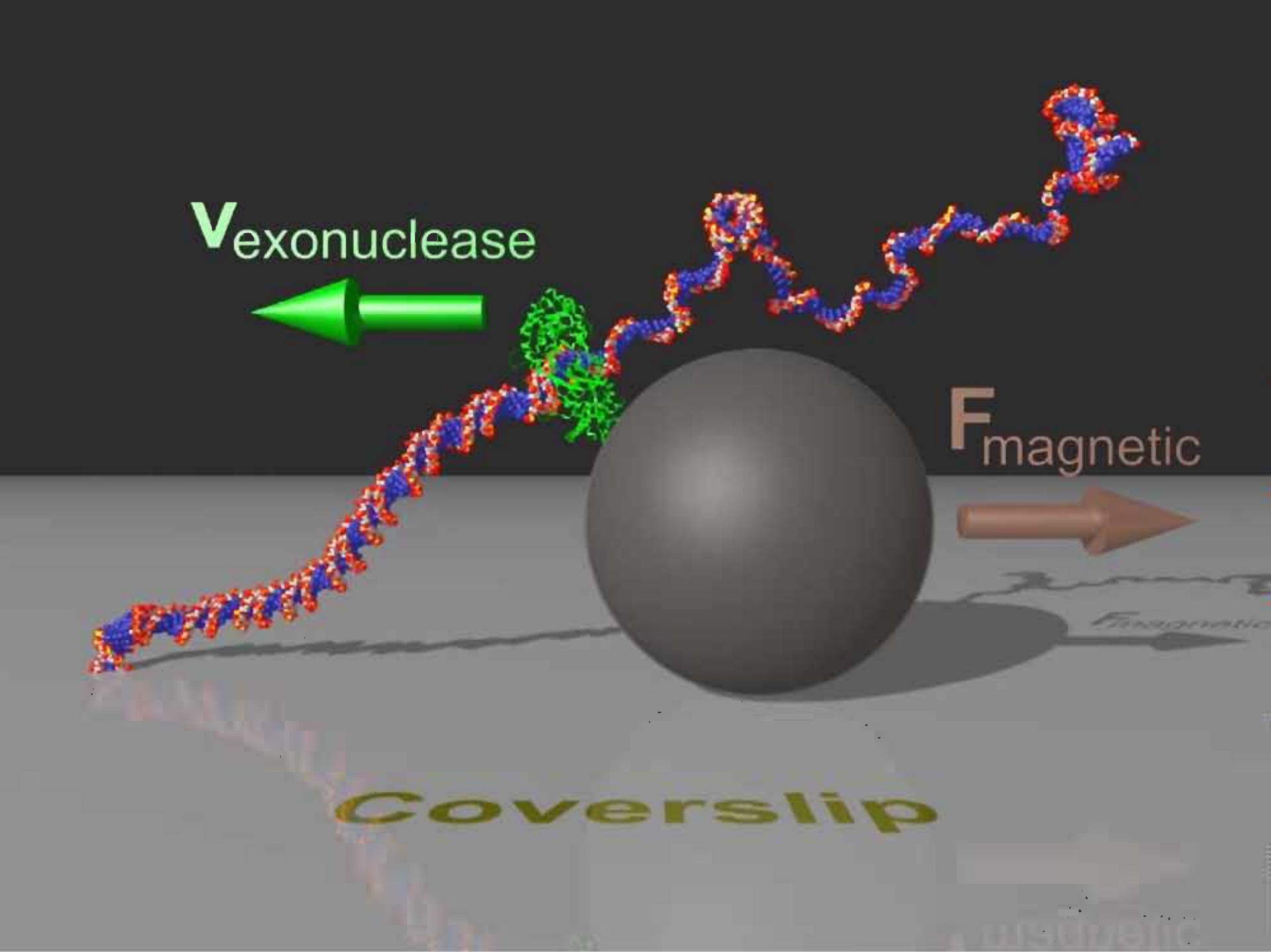


Figure from Kovall & Matthews, Science

λ Nuclease Active site

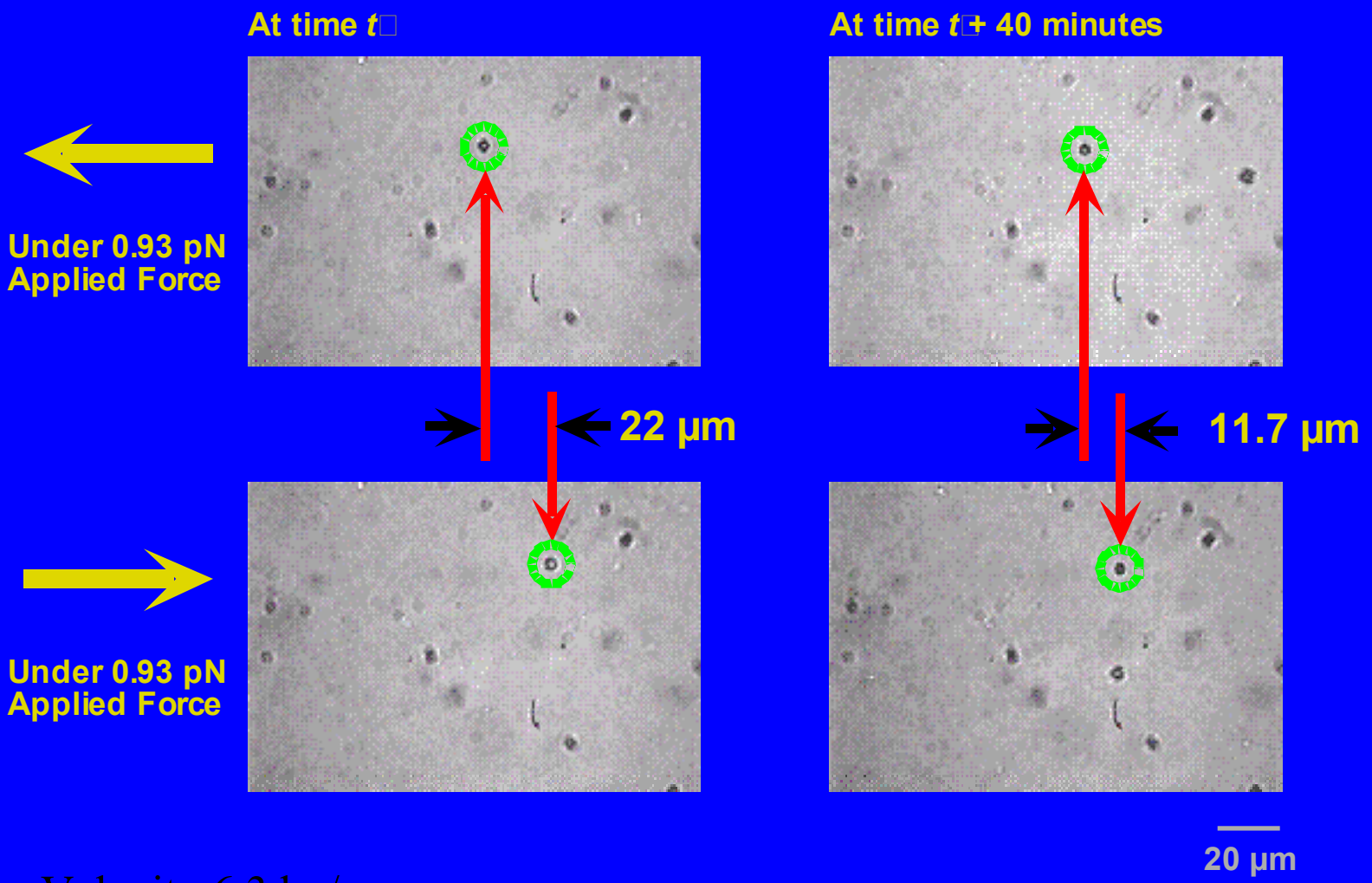


Figures from Kovall & Matthews PNAS 95, 7893 (1998)


Vexonuclease
Fmagnetic
Coverslip

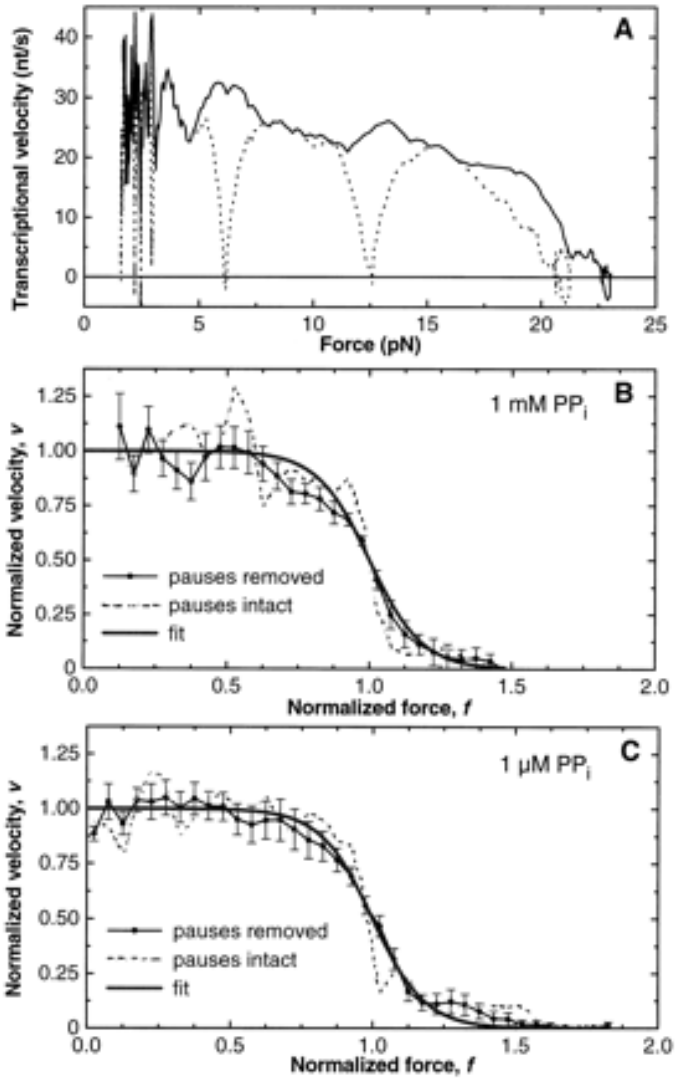
λ-Exonuclease No-Load Velocity and Processivity:

Wild Type Nonspecifically Absorbed On Nitrocellulose

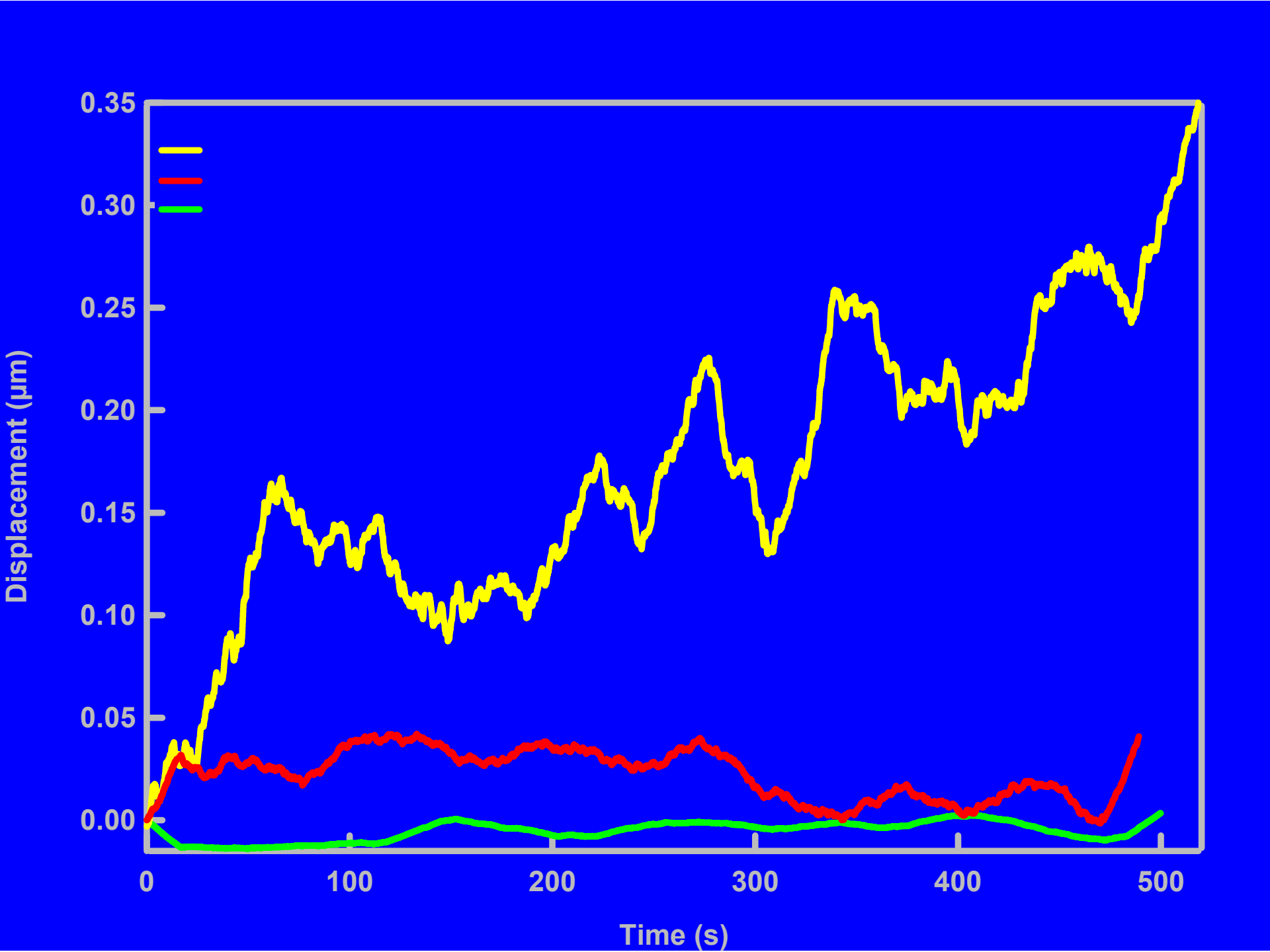


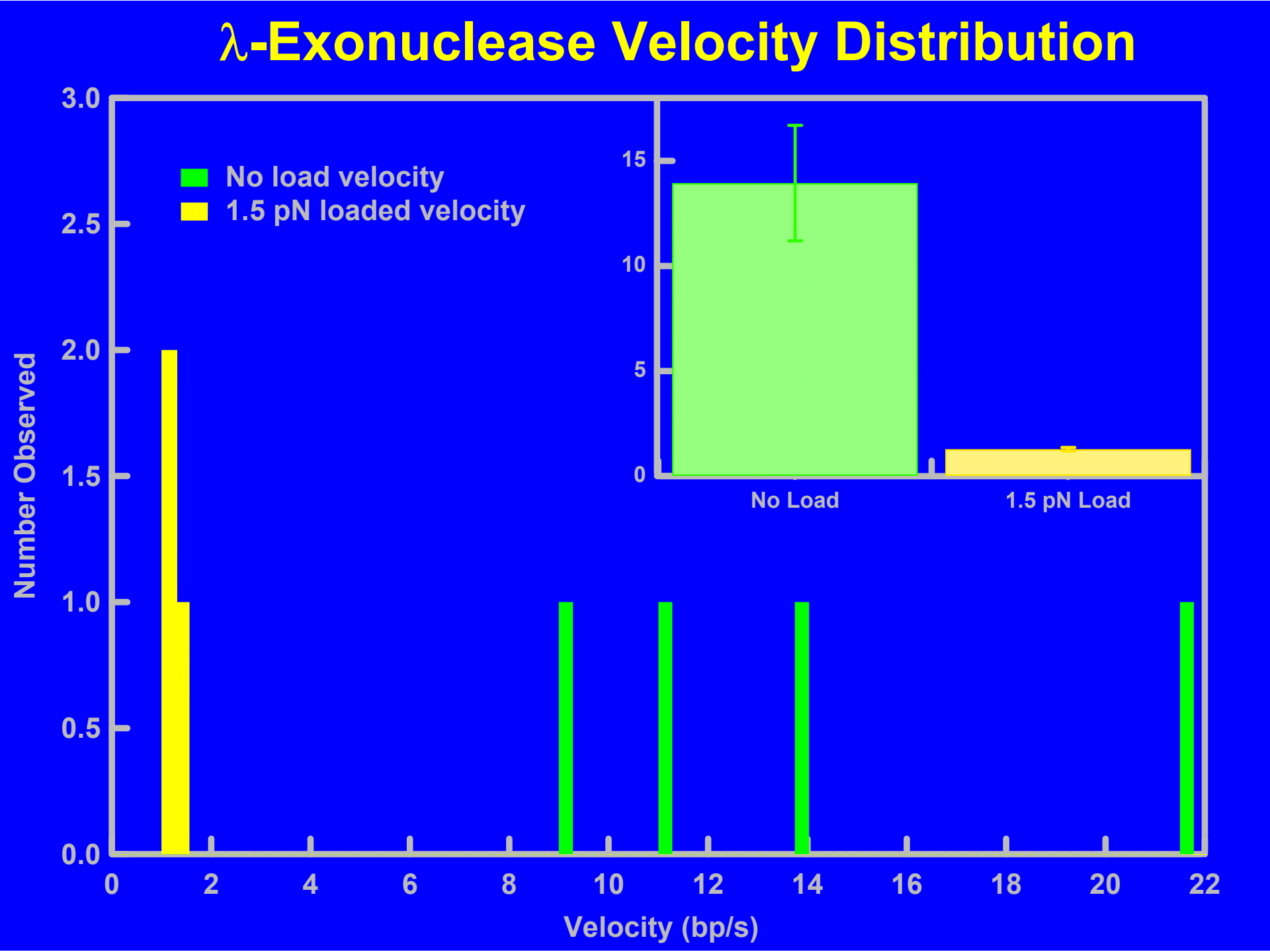
- Velocity 6.3 bp/sec
- Processive up to 15000 bp

Force - Velocity curve of a similar protein: RNA polymerase



Wong, Science,282,902, 1998


0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Displacement (μm)
0
100
200
300
400
500
Time (s)


λ-Exonuclease Velocity Distribution
No load velocity
1.5 pN loaded velocity
Number Observed
Velocity (bp/s)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
2
4
6
8
10
12
14
16
18
20
22
15
10
5
0
No Load
1.5 pN Load