Single molecule force spectroscopy

- Force apparatus
  - Optical tweezers
  - Magnetic tweezers
  - AFM

Reference:

KC Neuman & A Nagy, Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy. NATURE METHODS | VOL.5 NO.6 | JUNE 2008 | 491-505.

What is optical tweezers?

Optical Tweezers use light to manipulate microscopic objects as small as a single atom.

In the biological sciences, these instruments have been used to apply forces in the pN-range and to measure displacements in the nm range of objects ranging in size from 10 nm to over 100 μm.

First demonstration of optical tweezers

A. Ashkin, J.M. Dziedzic, J.E. Bjorkholm and S. Chu. 1986. "Observation of a Single-Beam Gradient Force Optical Trap for Dielectric Particles." Opt. Lett. 11 (5) 288-290.

The Nobel Prize in Physics 1997

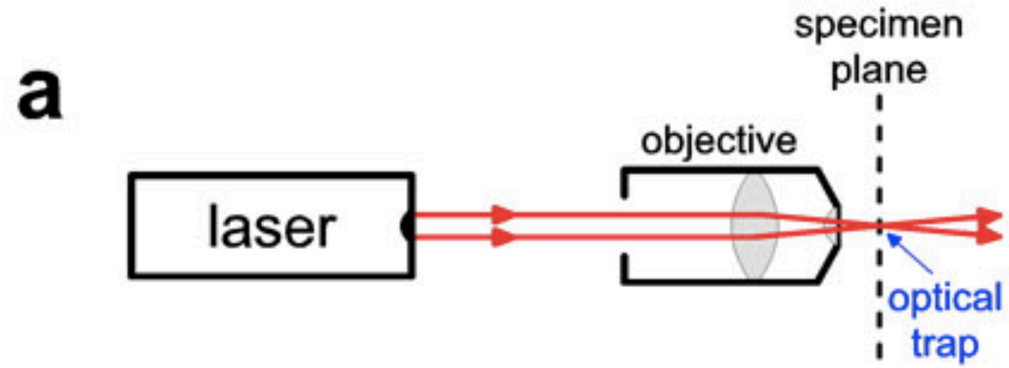
"for development of methods to cool and trap atoms with laser light"

Steven Chu

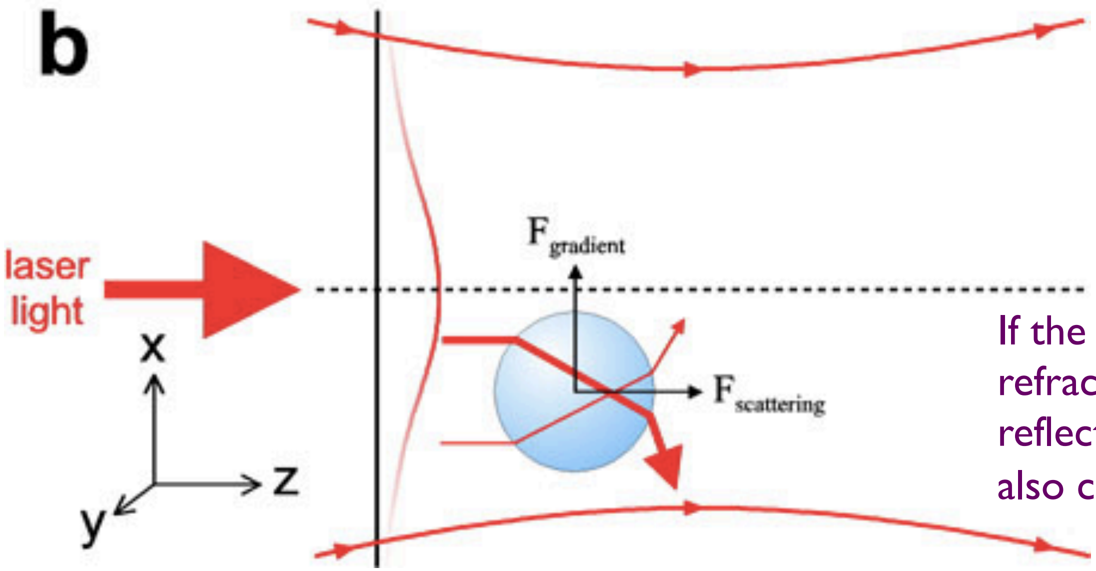
Claude Cohen-Tannoudji

William D. Phillips

Optical Tweezers–principles

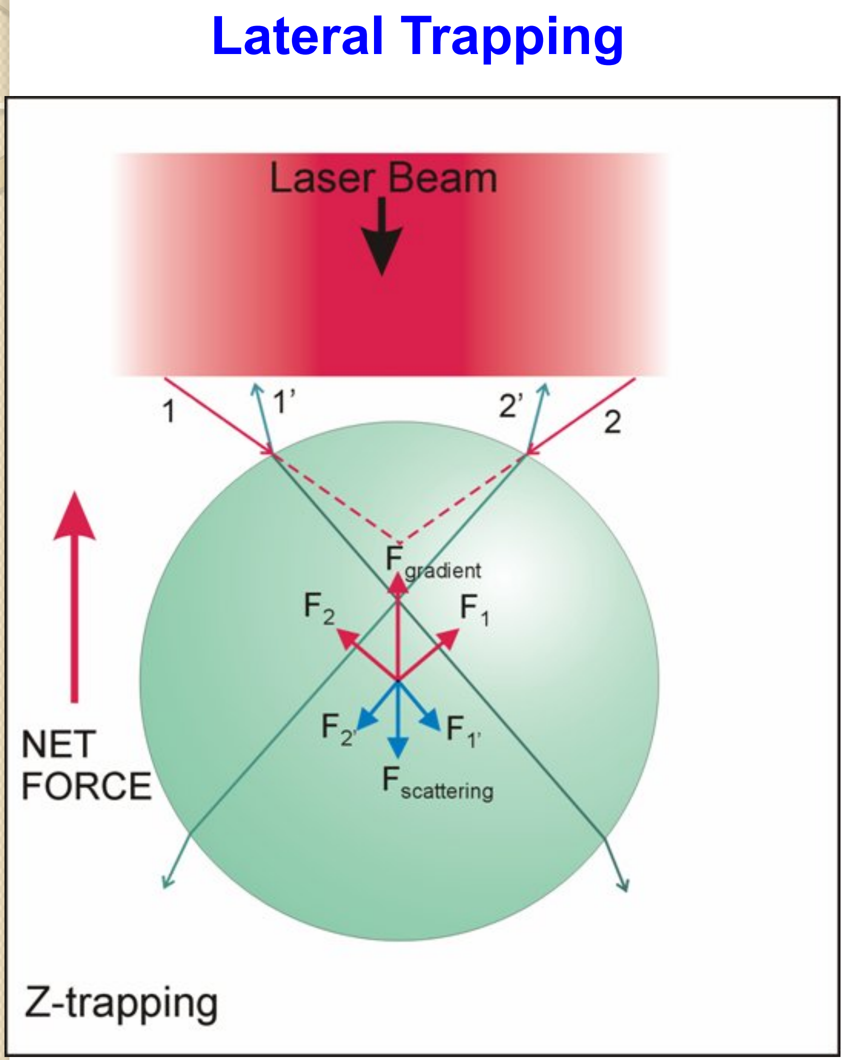


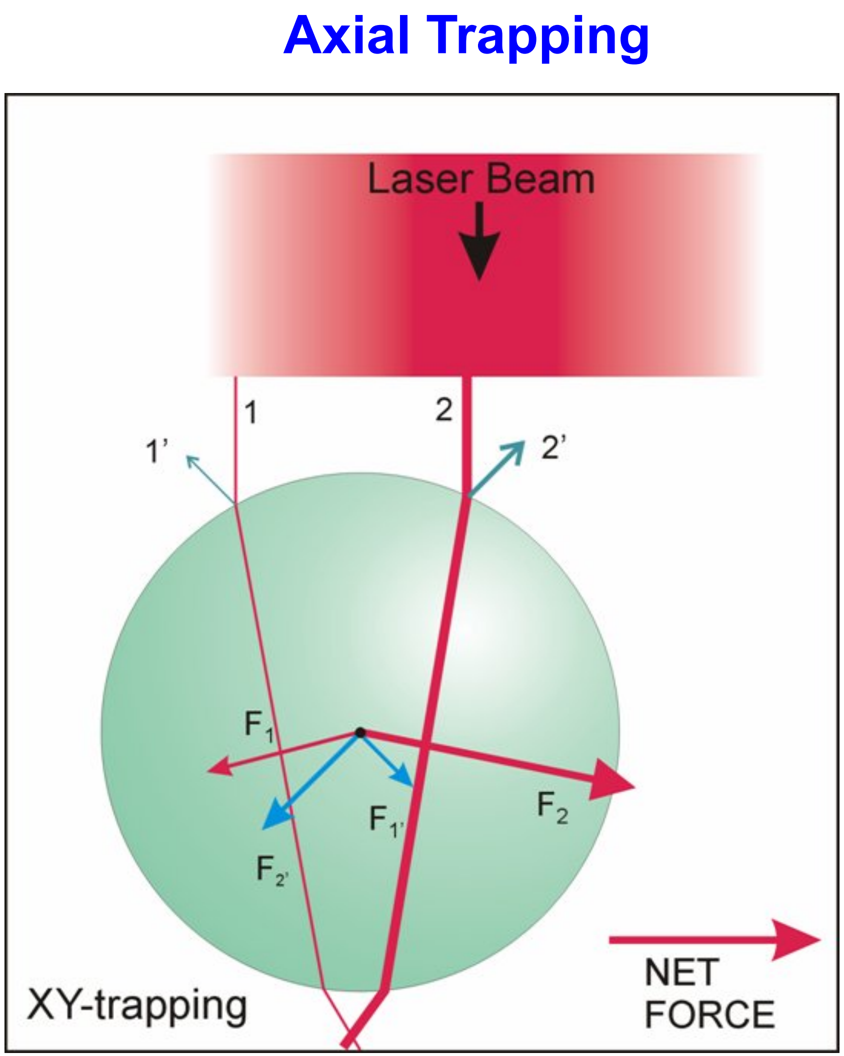
The $\mathbf{F}_{\mathbf{gradient}}$ is a restoring force that pulls the bead into the center.



If the contribution to $\mathbf{F}_{\mathbf{scattering}}$ of the refracted rays is larger than that of the reflected rays then a restoring force is also created along the z-axis.

Ray optics of momentum change

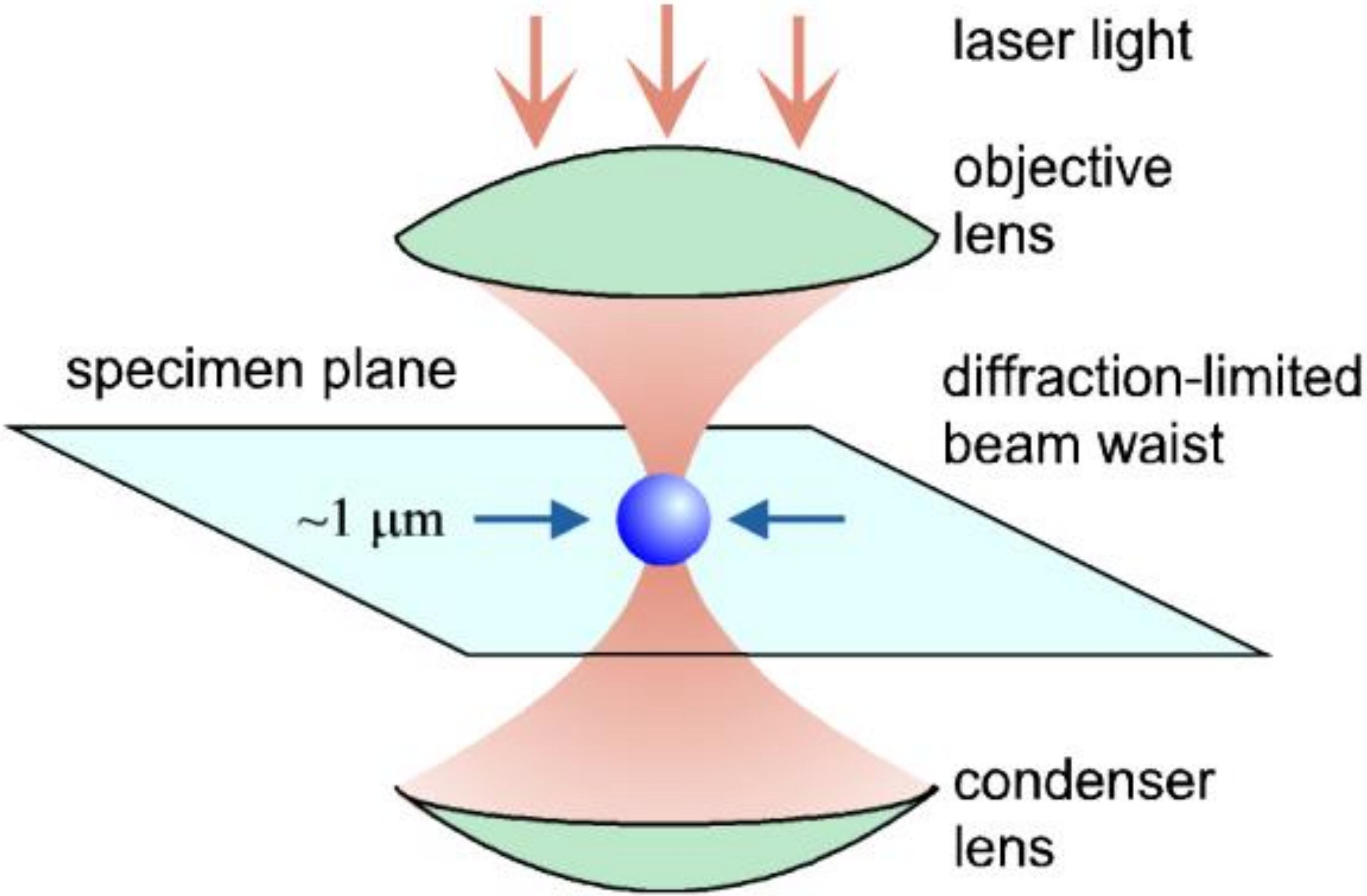




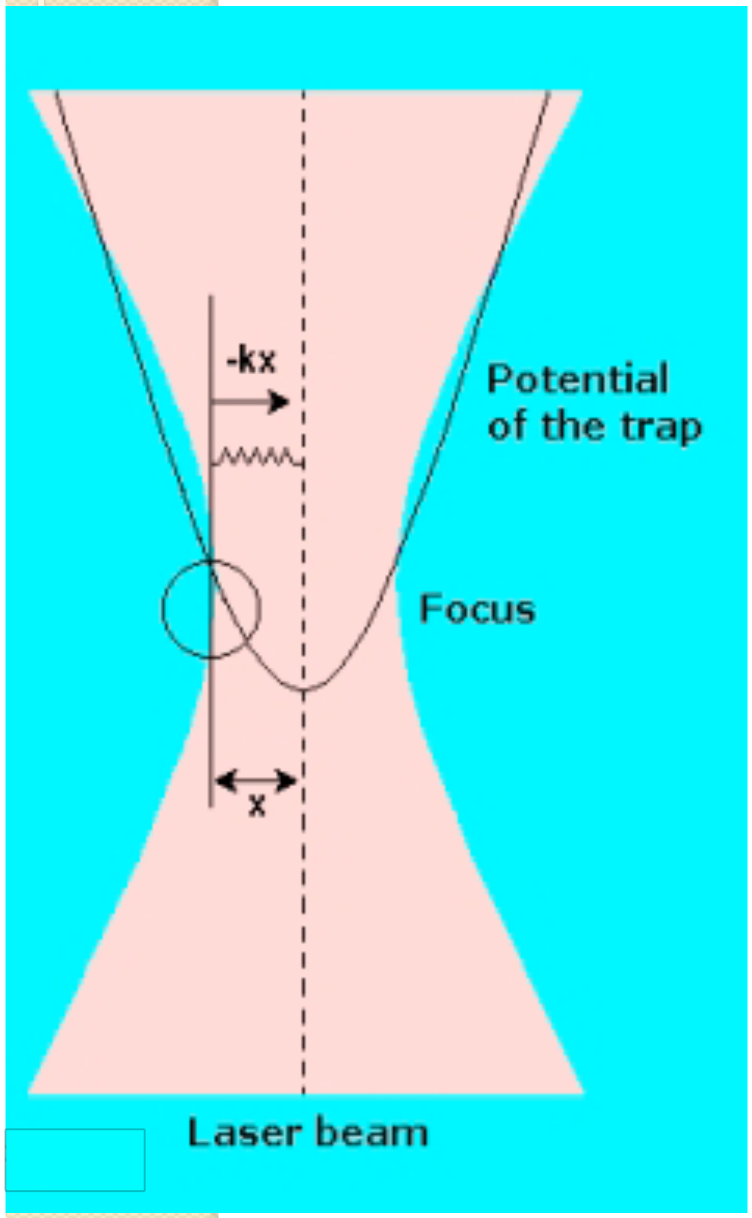
http://www.reading.ac.uk/physics/research/

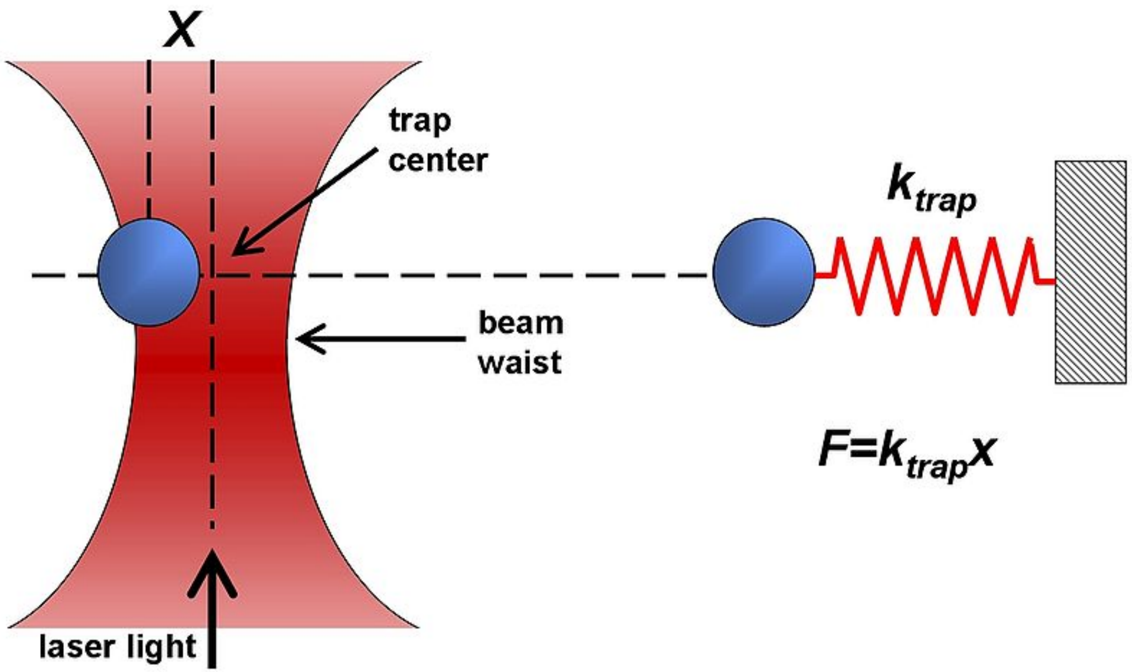
Optical tweezers

$$F_{grad} = \frac{1}{2}\nabla(\boldsymbol{p}\cdot\boldsymbol{E}) \propto \nabla(E^2)$$



Optical spring





Dielectrophoresis (DEP)

The classical DEP theory states that the dielectrophoretic force arises from the interaction of the induced dipole of a polarizable object and an external non-uniform electric field (Pohl 1978).

Potential energy of a dielectric object in an electric field:

$$U = -p \bullet E = -\alpha V E^2$$

Dielectrophoretic force: $F = -\nabla U \sim E \nabla E$

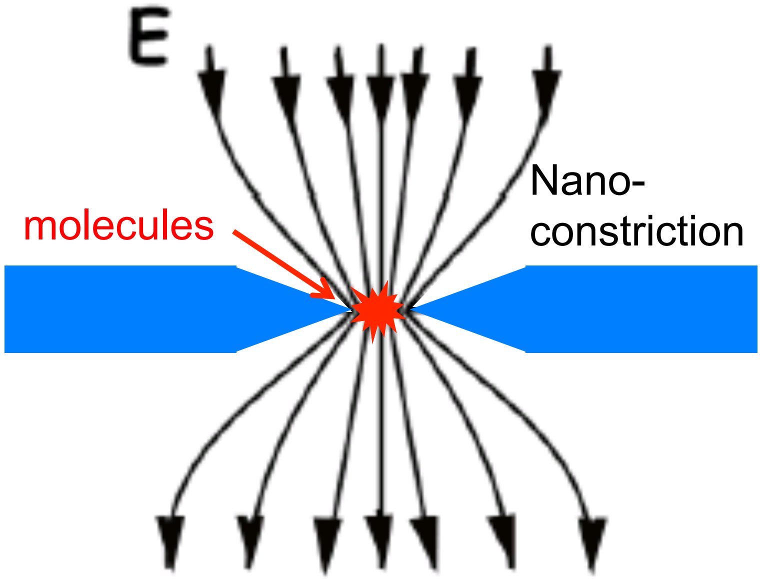
$$F = 2\pi a^3 \varepsilon_m \mathrm{Re}\left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right)\nabla(E^2)$$

Sphere of radius *a*, ε_m = 80 for water

Clausius-Mossotti factor

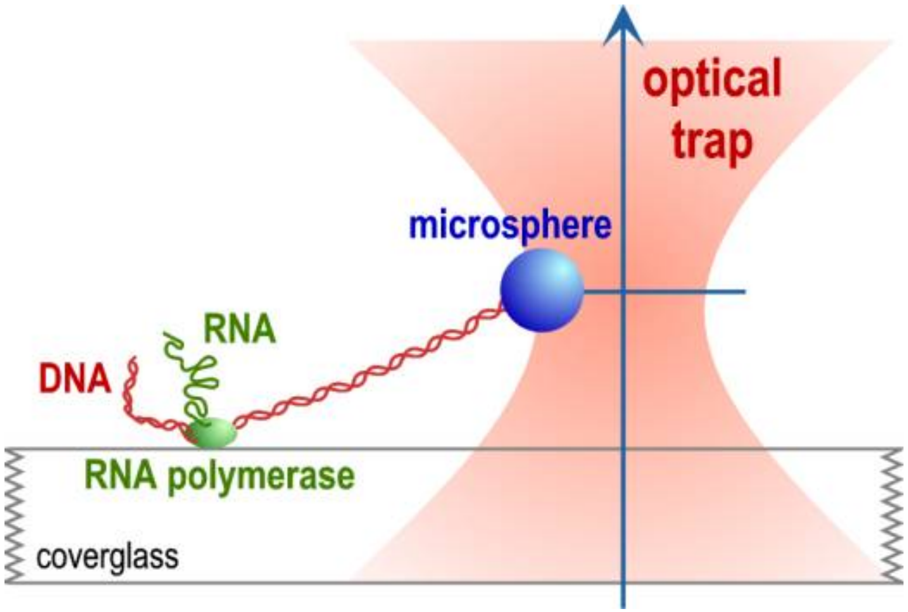
EDEP Molecular Trap vs. Optical Tweezers

EDEP molecular trap



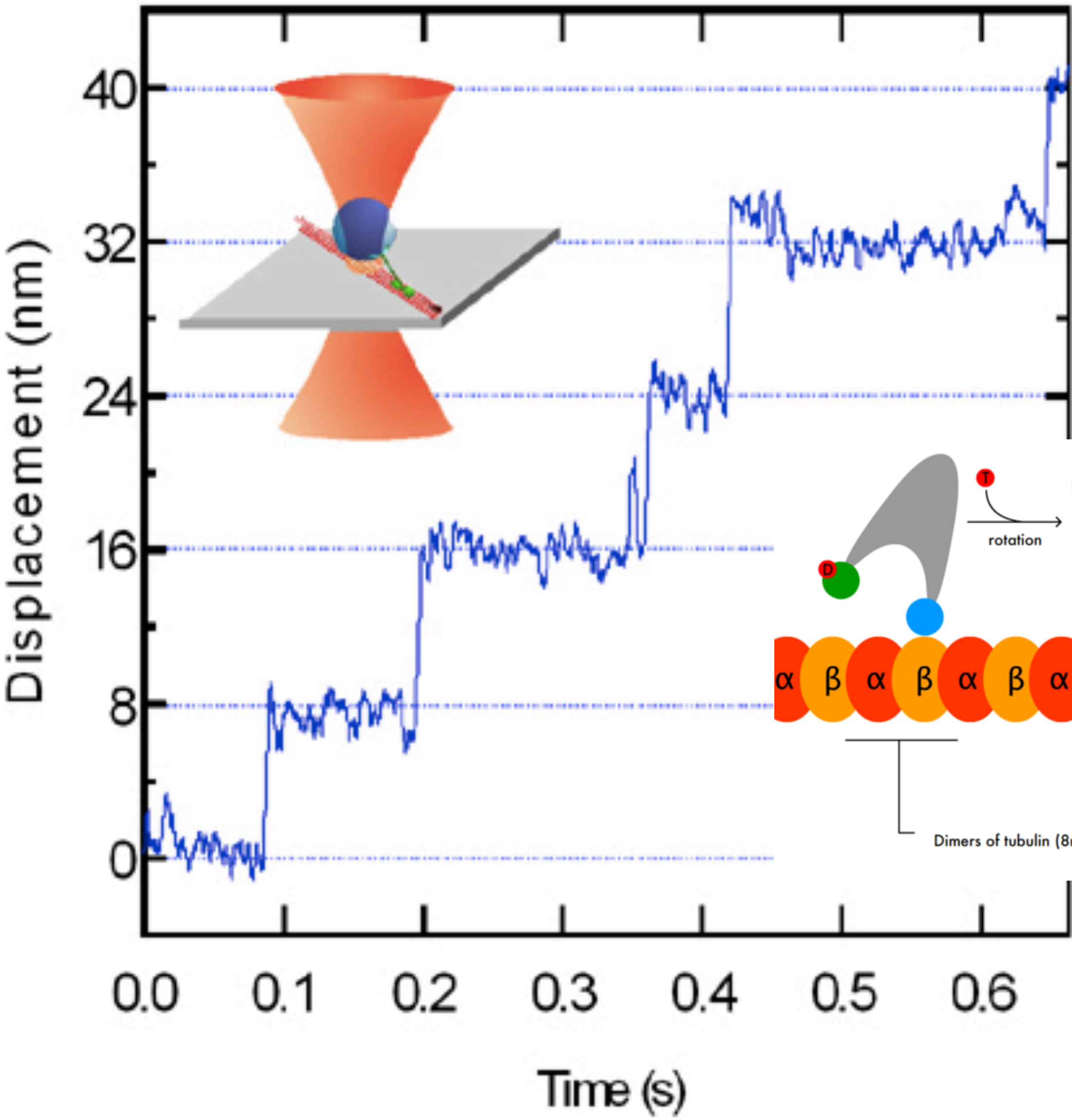
Electric field focused at the constriction

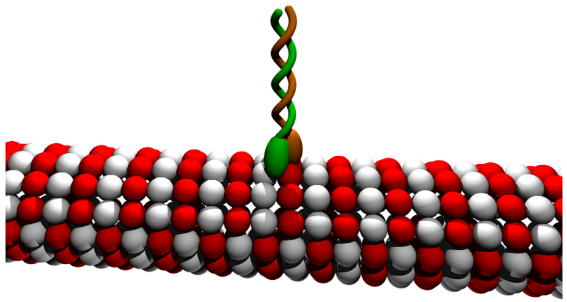
Optical trap



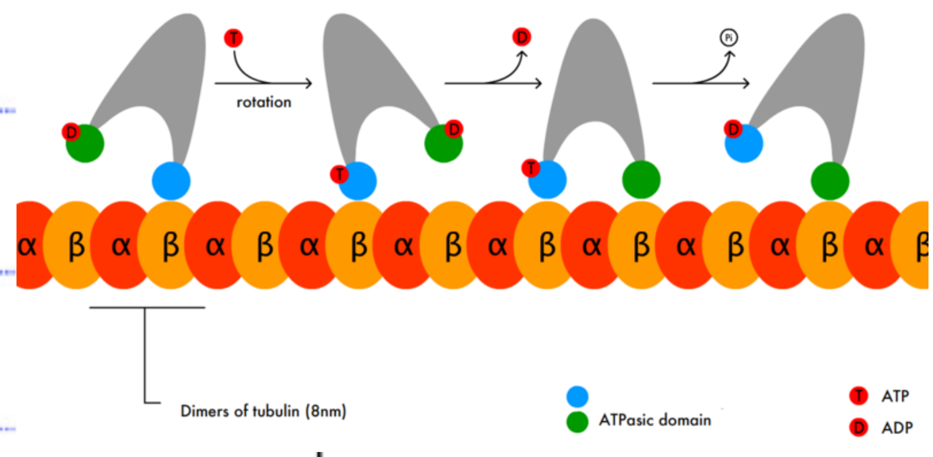
Electric field focused at the focal point

The 8-nm steps of kinesin along microtubule against a 5-pN force





Motility of kinesin



[http://www.stanford.edu/group/blocklab/ Optical Tweezers Introduction.htm](http://www.stanford.edu/group/blocklab/Optical%20Tweezers%20Introduction.htm)

The 'dumbbell' experiment

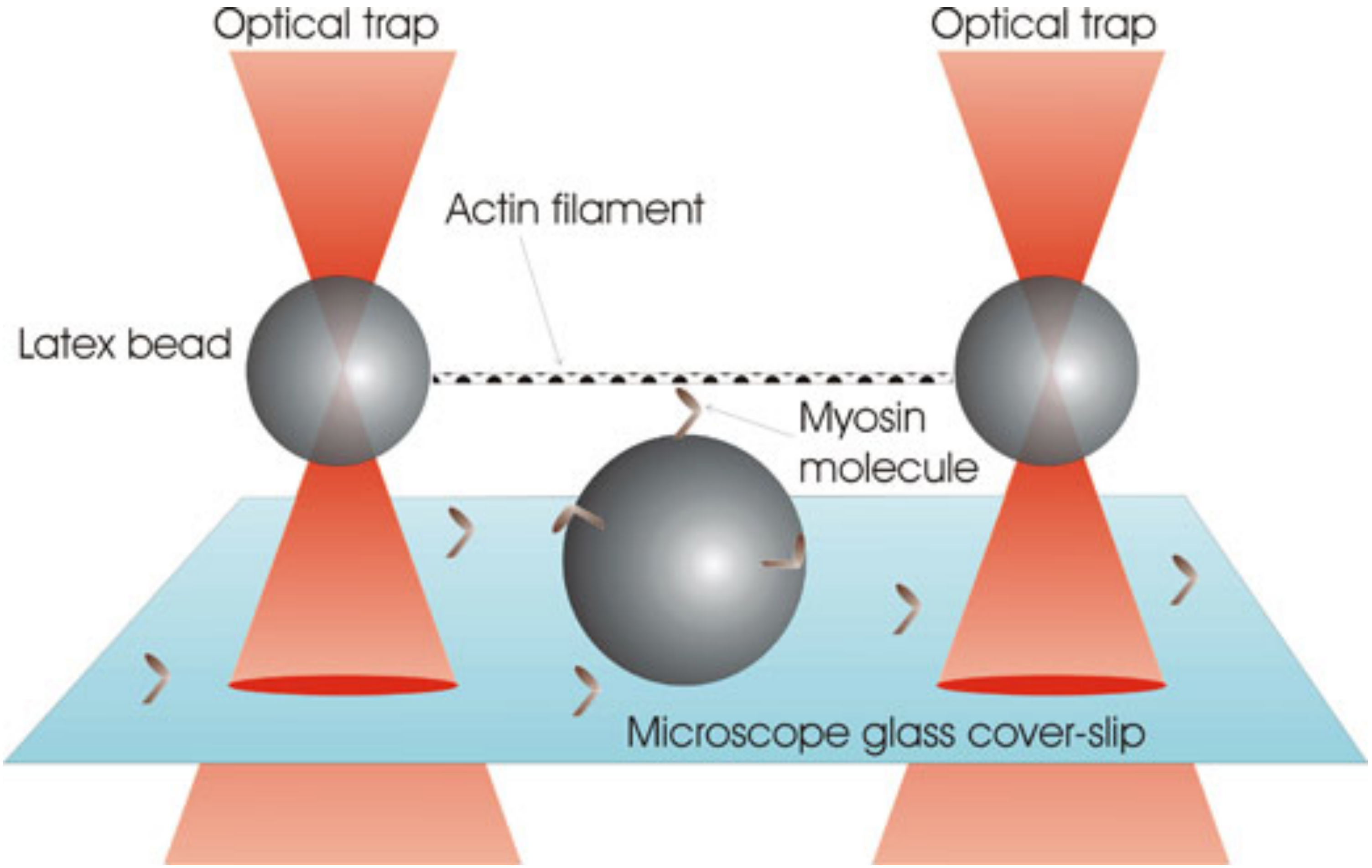
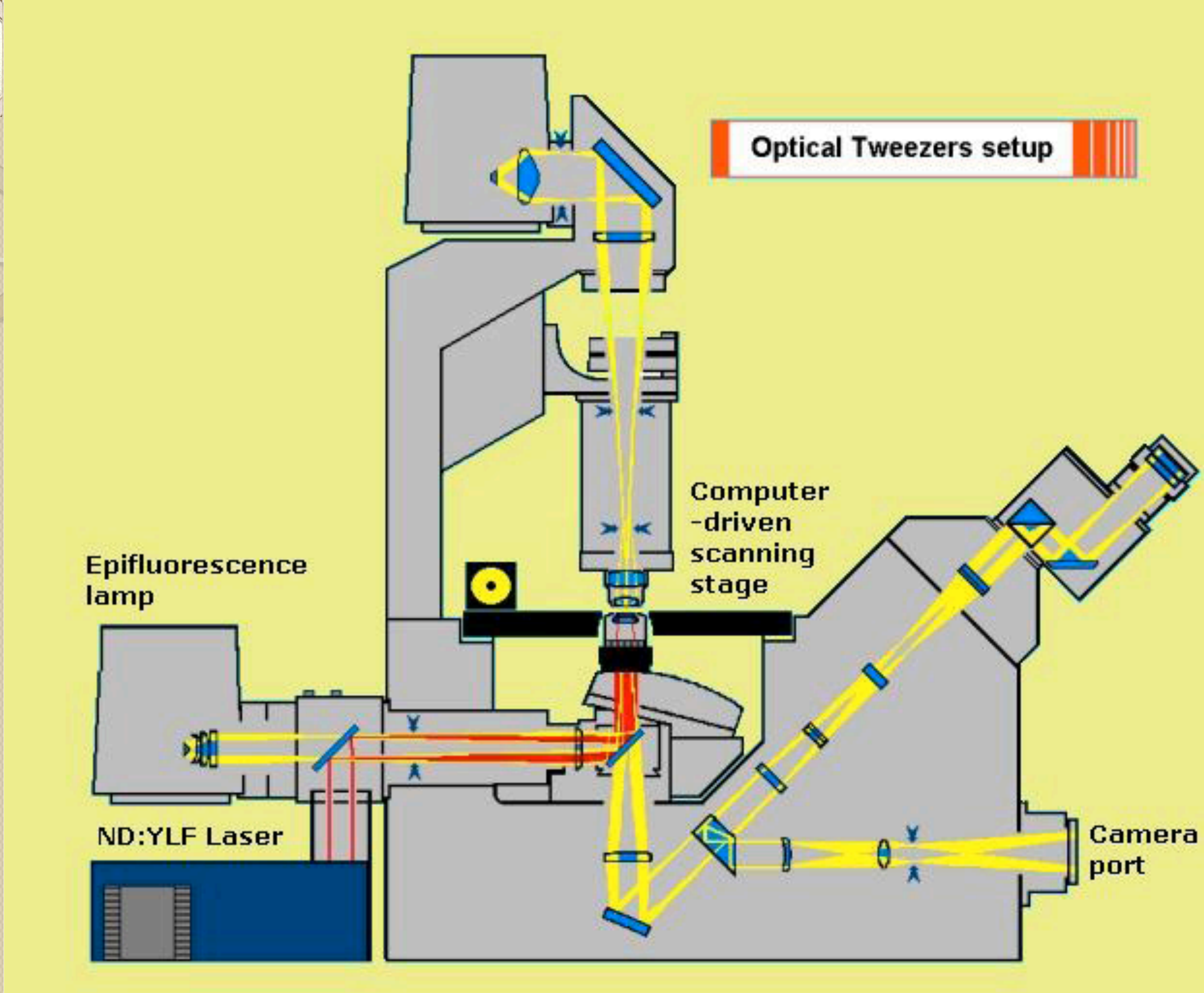
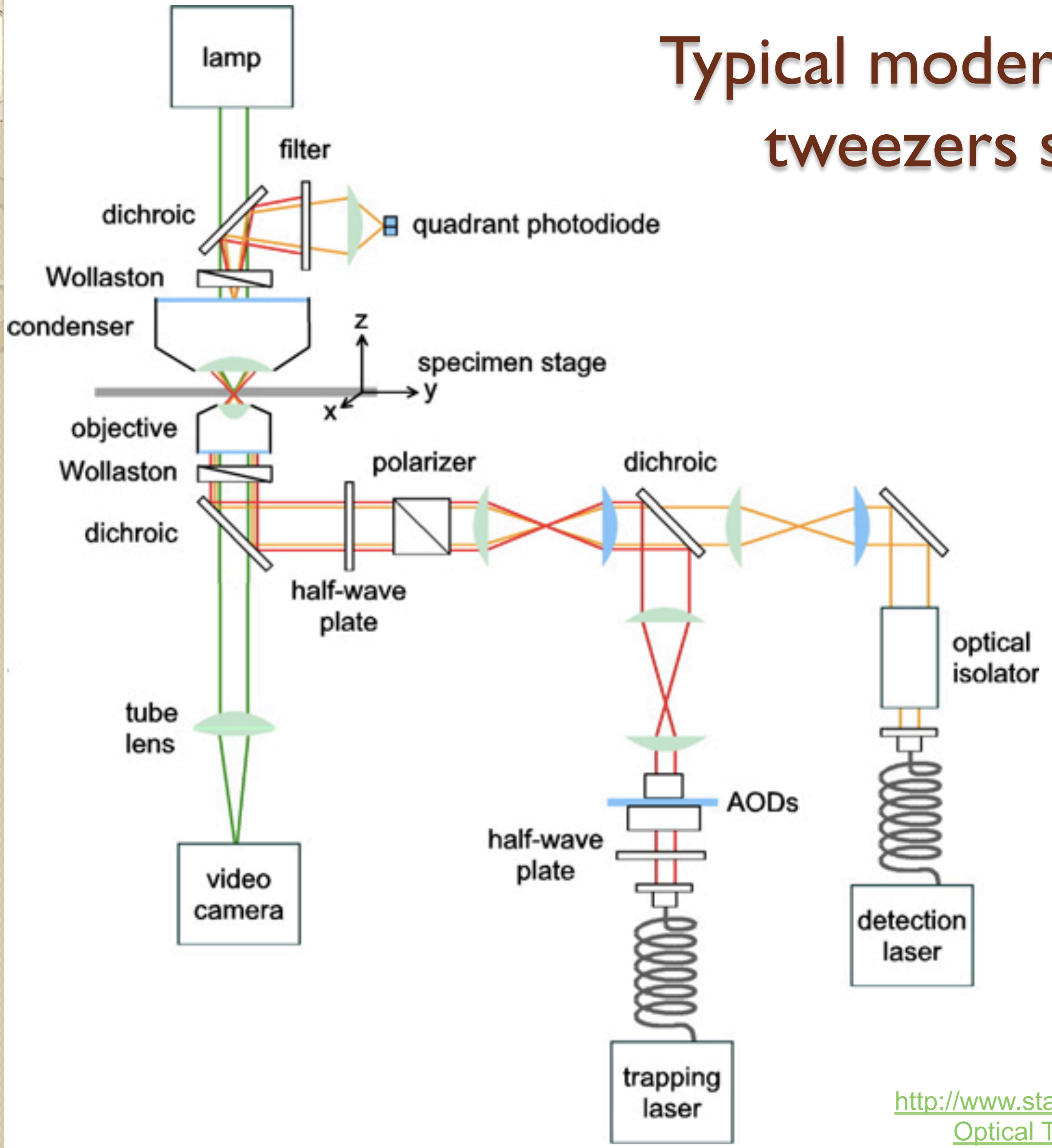


Image courtesy of Alexandre Lewalle



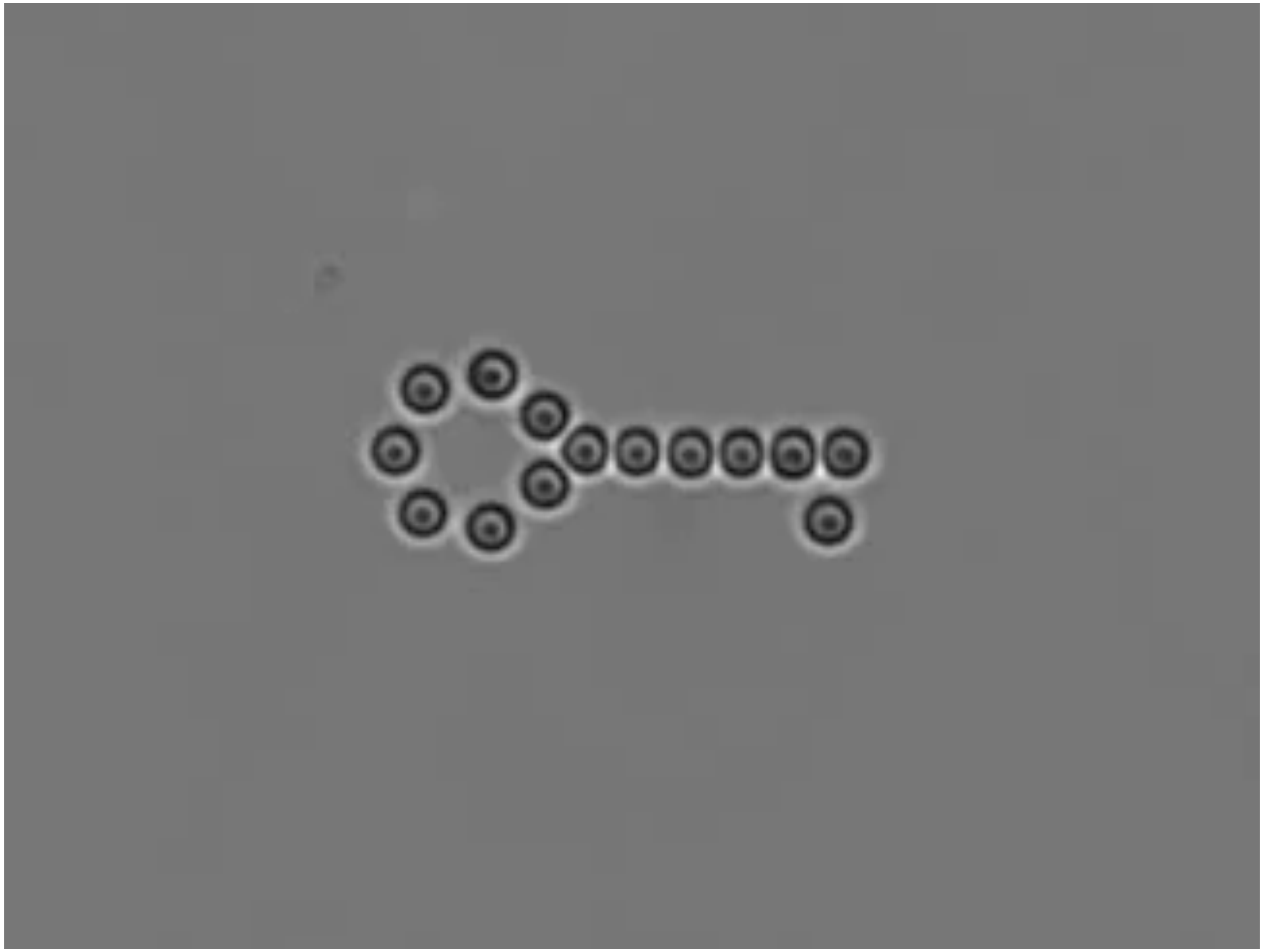
http://www.fz-juelich.de/isb/isb-1//enderlein/francesco/Page4.html

Typical modern optical tweezers setup



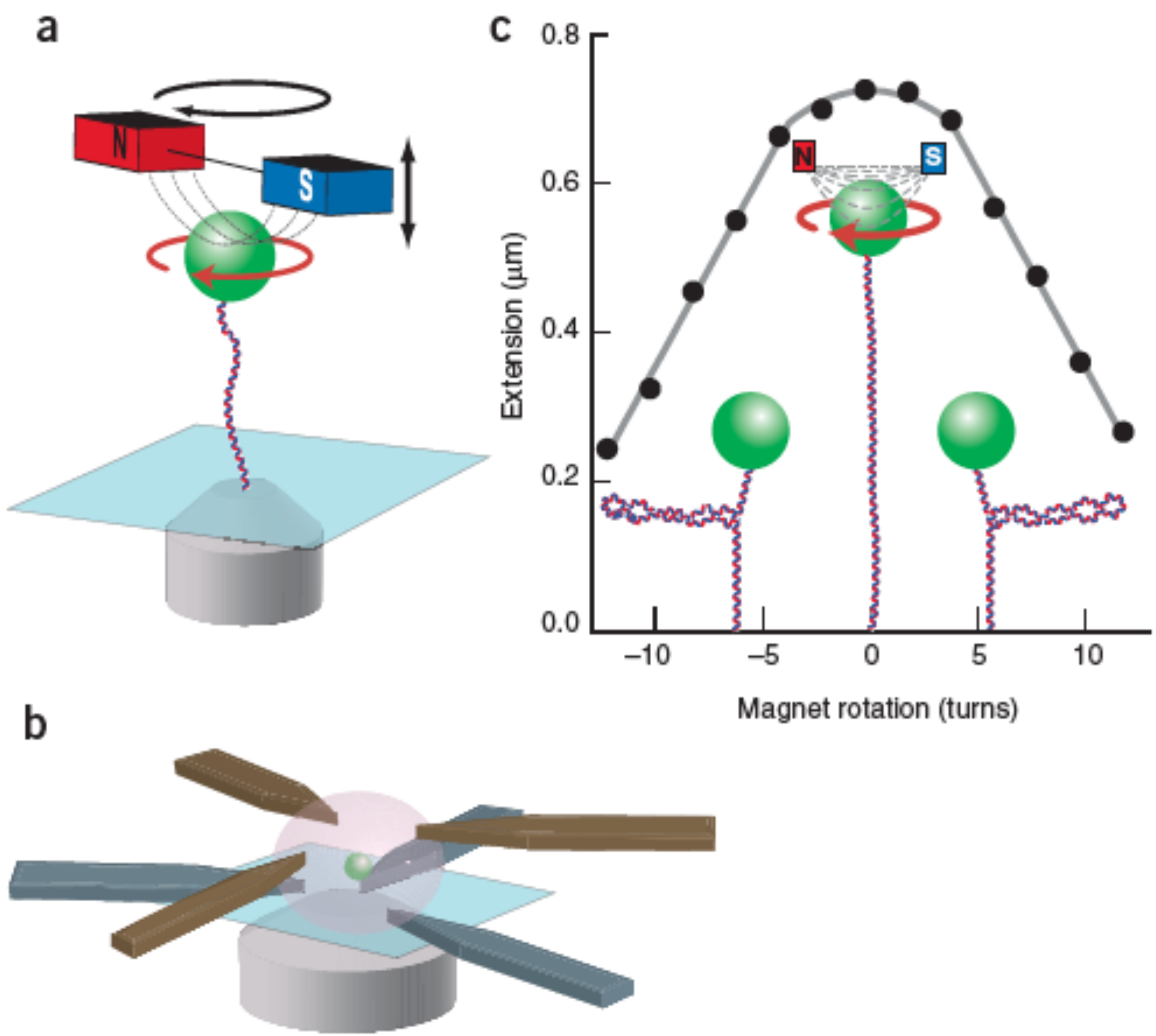
http://www.stanford.edu/group/blocklab/Optical Tweezers Introduction.htm

Optical Tweezers in Action



http://www.youtube.com/watch?v=ju6wENPtXu8

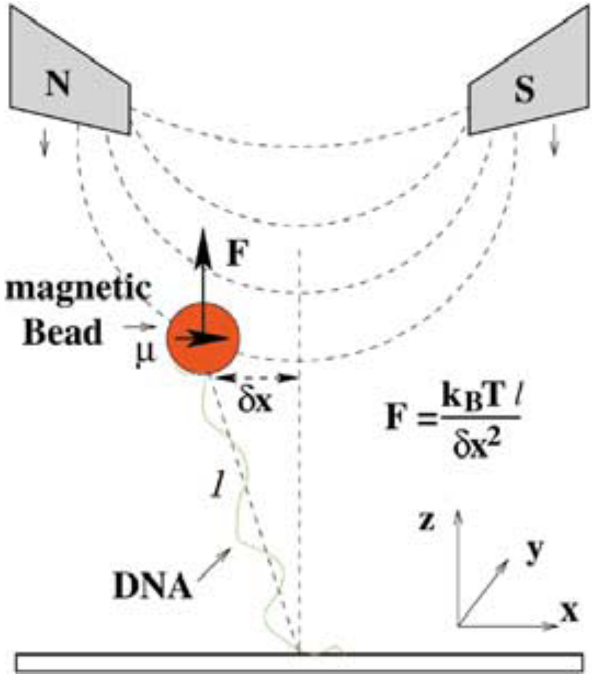
Magnetic Tweezers for Single Molecule Manipulation



Theory of Magnetic Tweezers

$F_M = \nabla(\mathbf{m} \cdot \mathbf{B}) = \nabla(mB)$

$$F_{magnetic} = m_{sat} \frac{\partial B}{\partial z} = M_{max} V \frac{\partial B}{\partial z}$$



Assumption:

Magnetic moment of a super-paramagnetic bead having a saturated value

4.5-µm diameter bead →

2.8-µm diameter bead → $m_{sat} \approx 1.42 \times 10^{-13}\ Am^2$

1.0-µm diameter bead → $m_{sat} \approx 2.25 \times 10^{-14}\ Am^2$

$$\Rightarrow F_{magnetic} \propto \frac{\partial B}{\partial z}$$

As the field gradient ∼100 T/m

4.5-µm diameter bead ⇒ $F_{mangetic}$: **> 60 pN**

2.8-µm diameter bead ⇒ $F_{mangetic}$: **∼ 14 pN**

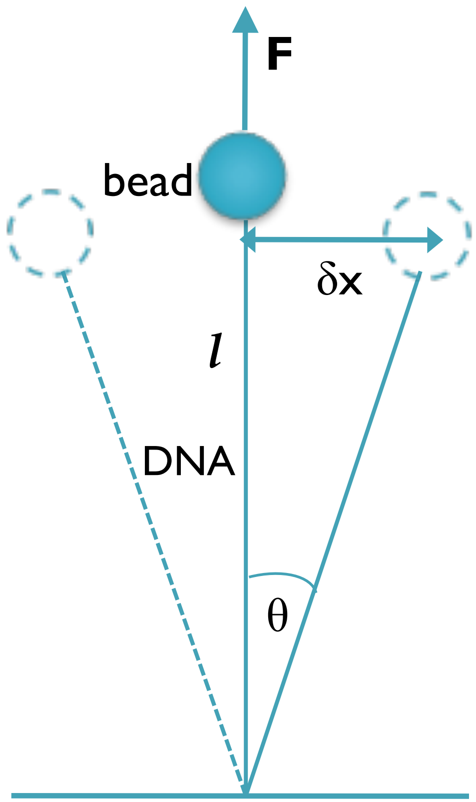
1.0-µm diameter bead ⇒ $F_{mangetic}$: **∼ 2 pN**

Force Calibration

- Calibration against flow field ($F=6\pi\eta rv$)
- AFM cantilevers can be calibrated using a set of levers of decreasing stiffness
- Trap stiffness in all cases easily determined by analyzing Brownian motion

Brownian Motion Analysis

(Tethered bead in a harmonic potential)



$$F_x = F\sin\theta \sim F\theta \sim F\frac{\delta x}{l} = -k_x \delta x$$

Equipartition of energy:

$$\frac{1}{2}k_x < \delta x^2 > = \frac{1}{2}k_B T$$

$$\frac{F}{l} < \delta x^2 > = k_B T$$

$$F = \frac{k_B T l}{< \delta x^2 >}$$

Magnetic Tweezers Setup

1. Magnetic tweezers configuration by Gauss meter

2. Magnetic force calibration by viscous drag force

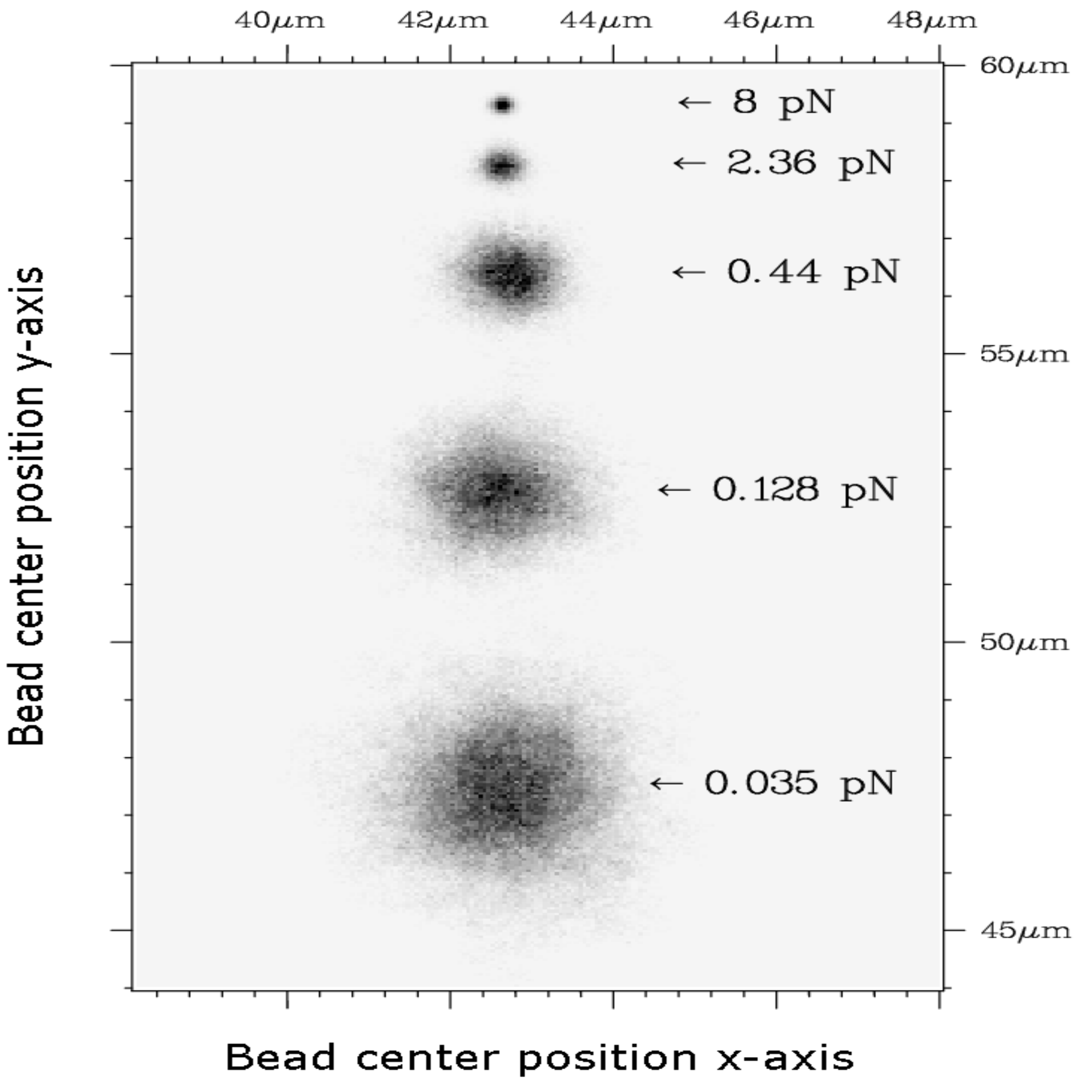
Stokes' law $$F_{magnetic} = 6\pi\eta r v_f$$

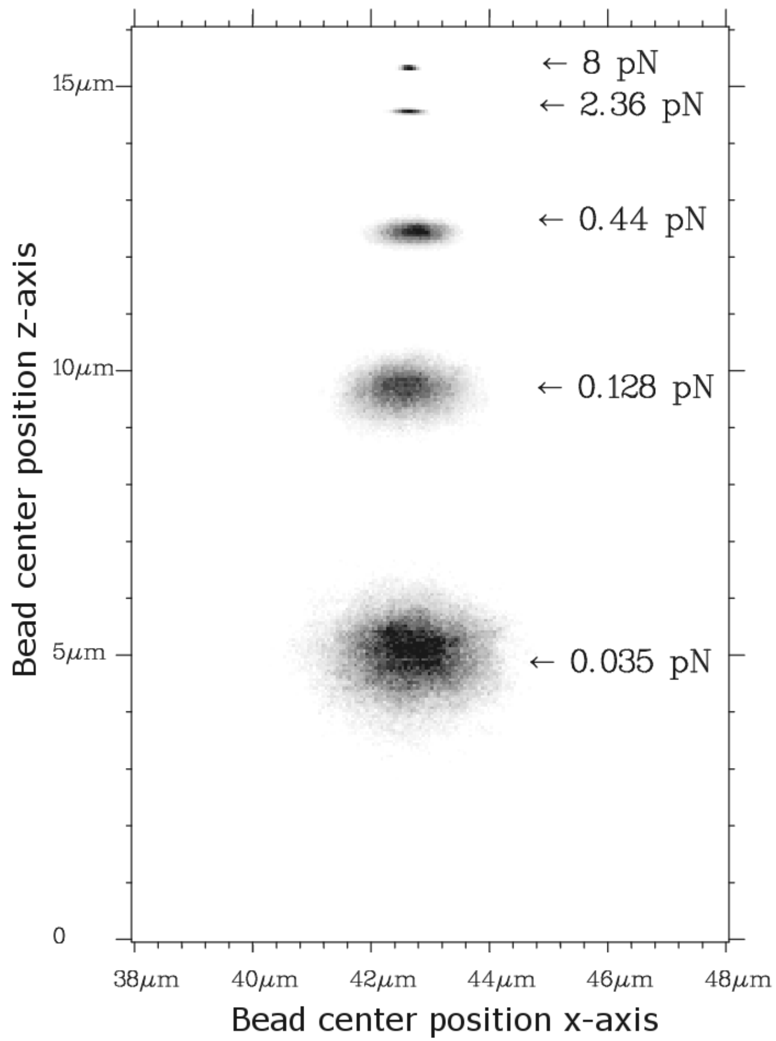
3. Force measurement for single molecule by using particle tracking algorithm

Equipartition theorem $$F = \frac{k_B T l}{< \delta x^2 >}$$

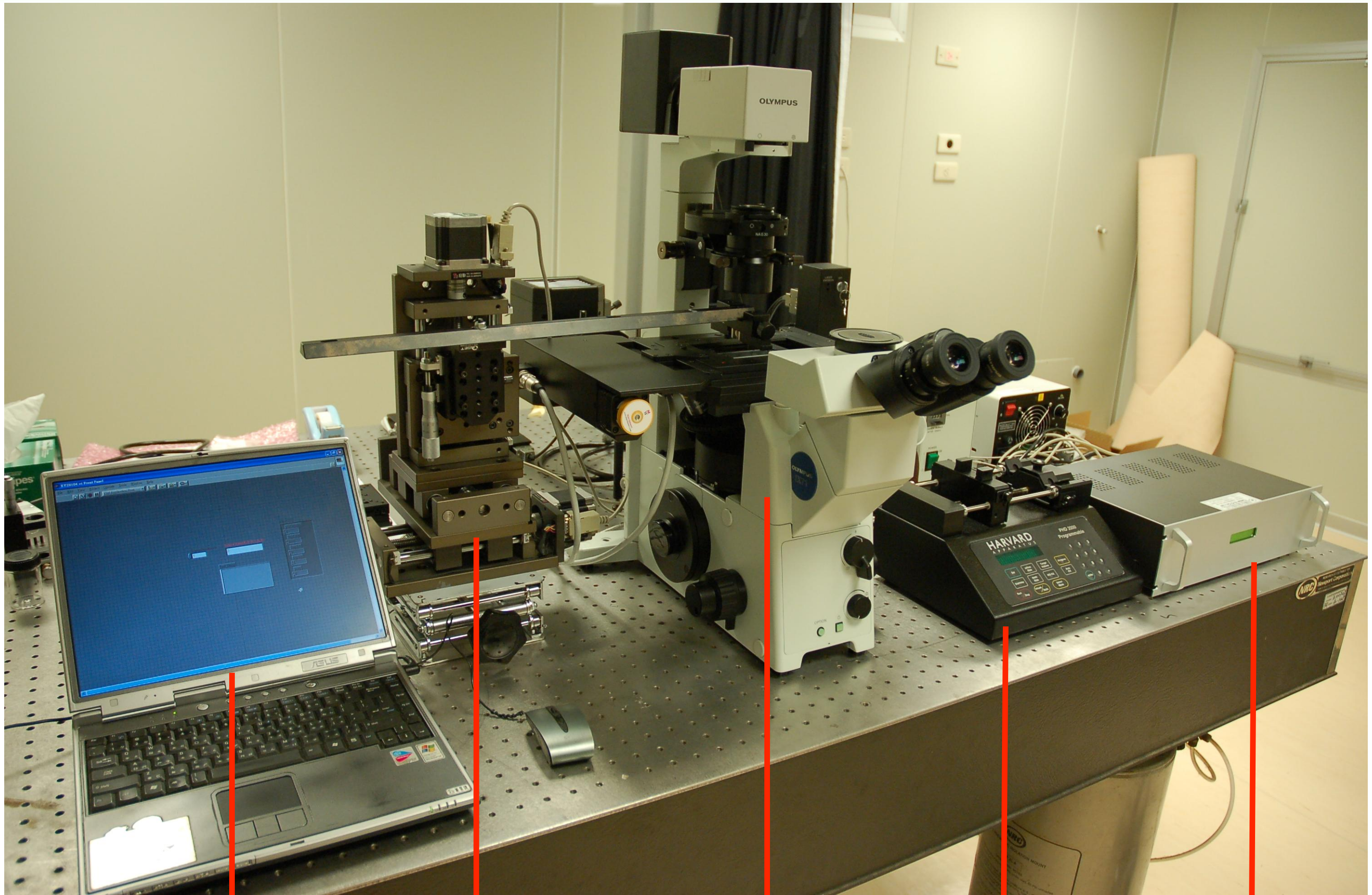
Force	Relative extension	RMS of fluctuation
10 fN	0.083	~ 815 nm
0.1 pN	0.5	~ 632 nm
1 pN	0.86	~ 262 nm
10 pN	0.97	~ 88 nm
100 pN	~2	~ 40 nm

Excellent low-force measurement technique





Experimental Setup



LabView Control Panel

3-axis Stage

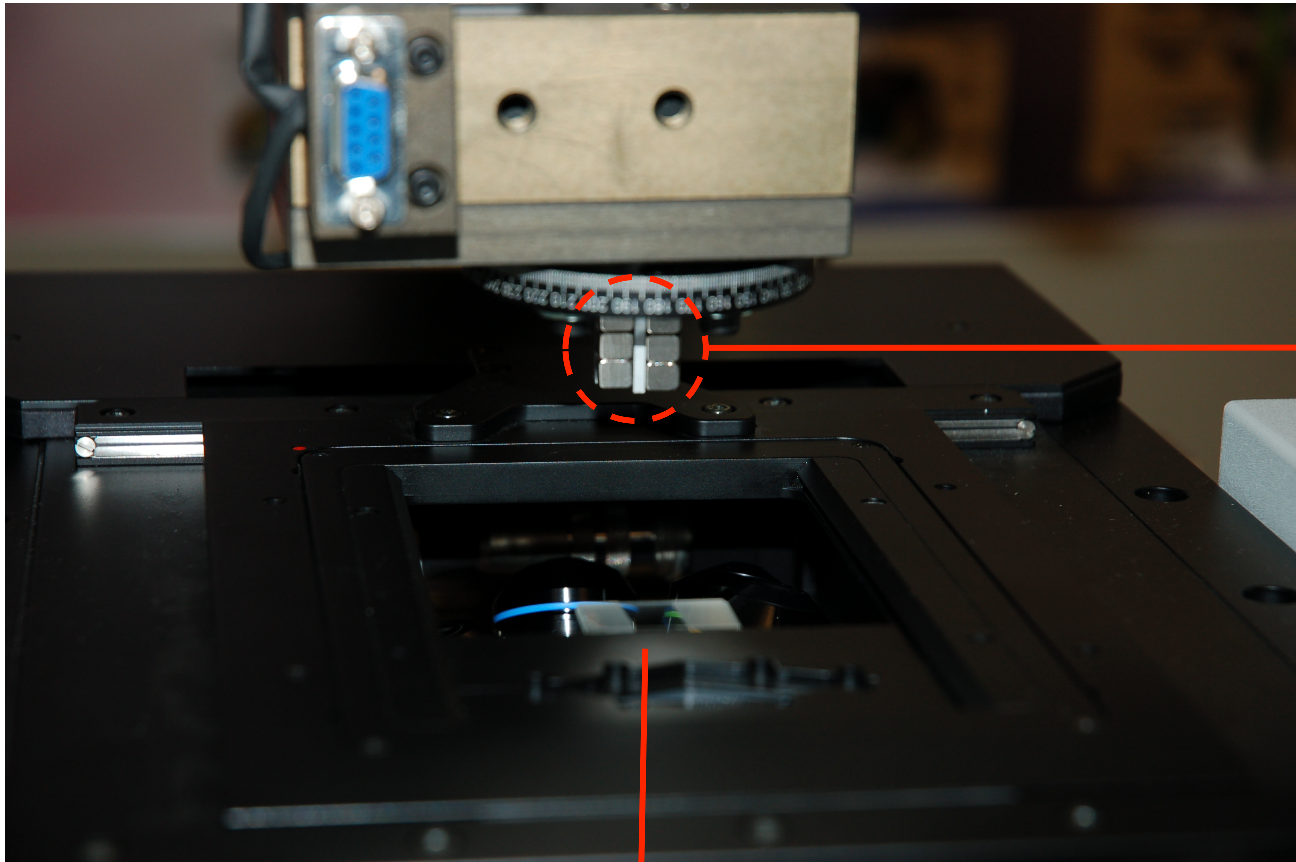
Inverted Microscope

Syringe Pump

Control Box

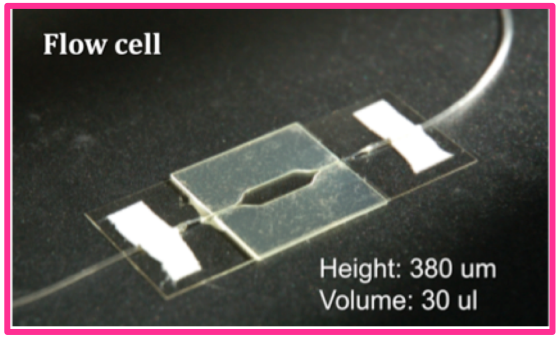
Nanobioscience Lab

Experimental Setup

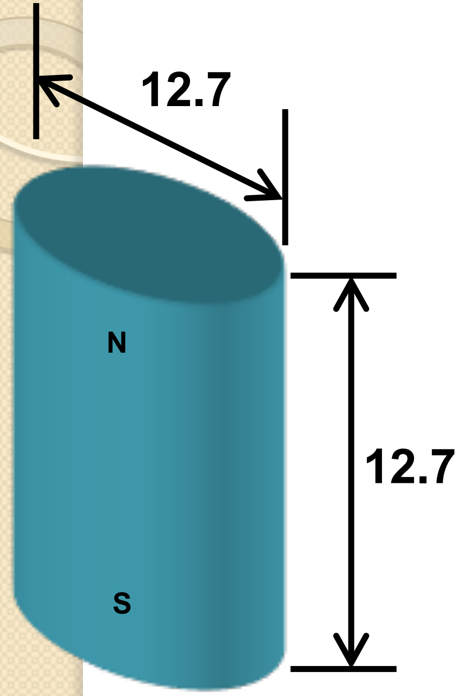


Permanent Magnets

Microfluidic chip

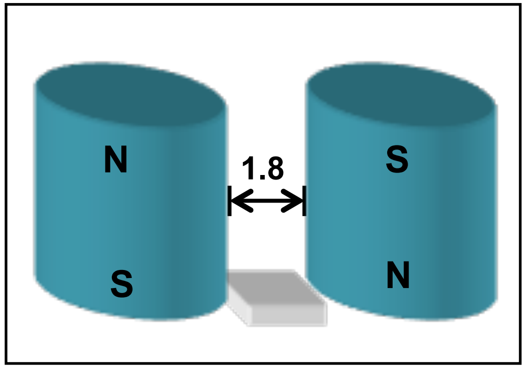


Experimental Parameters



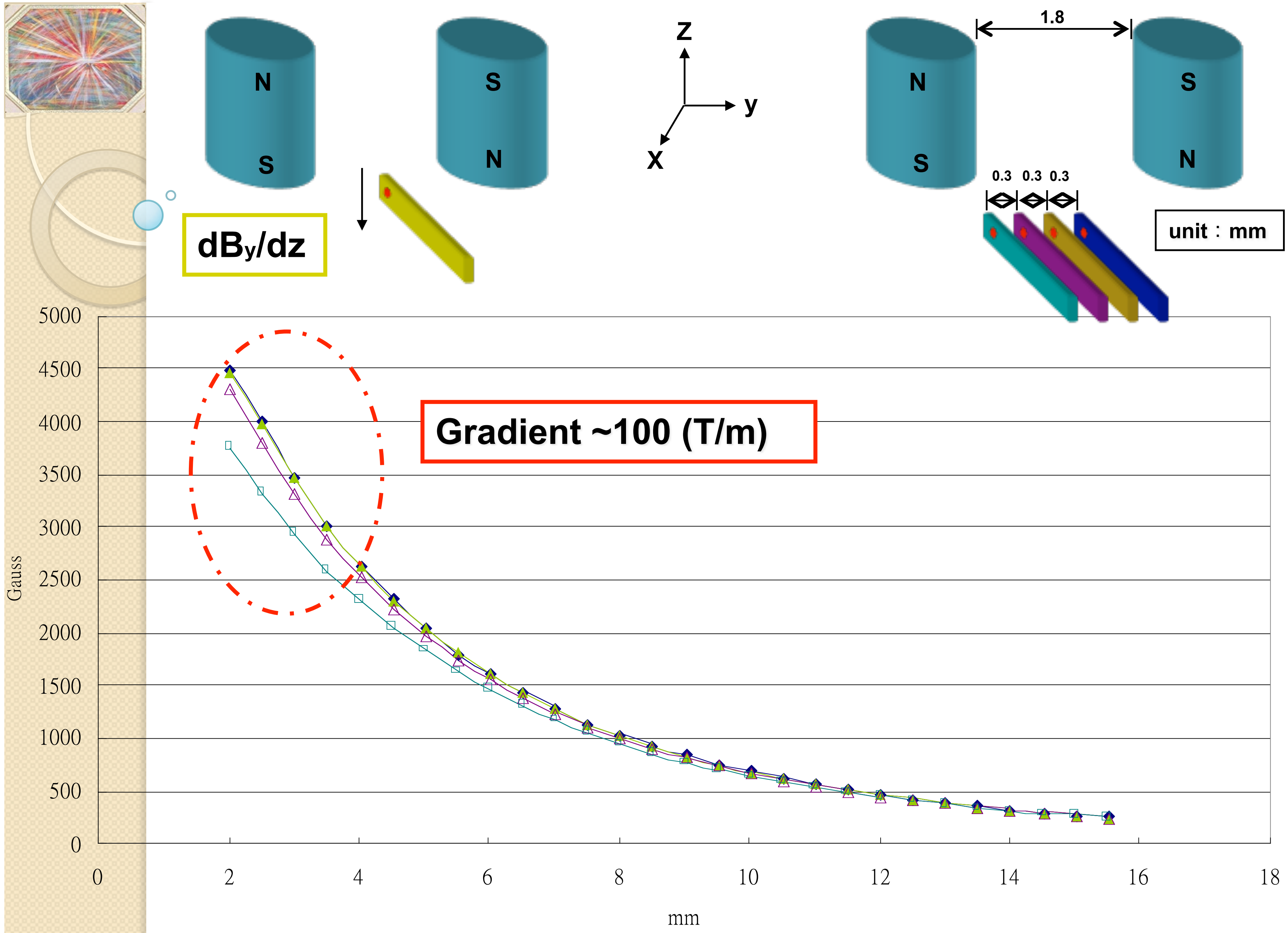
Permanent Magnet

Permanent Magnet	Neodymium Iron Boron
Material	NdFeB
Diameter	12.7mm
Height	12.7mm
Gap	1.8mm
Maximum magnetic filed	5490 Gauss



unit：mm

Gauss Meter



N
S
S
N
dBy/dz
Z
y
X
1.8
0.3 0.3 0.3
unit : mm
Gradient ~100 (T/m)
Gauss
5000
4500
4000
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0
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6
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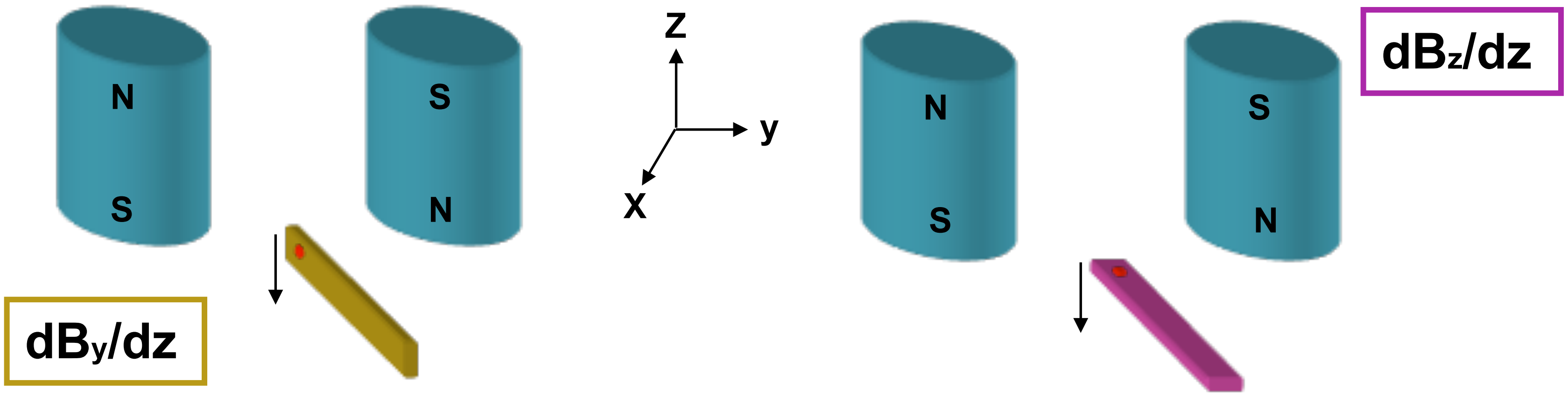
16
18
mm


N
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S
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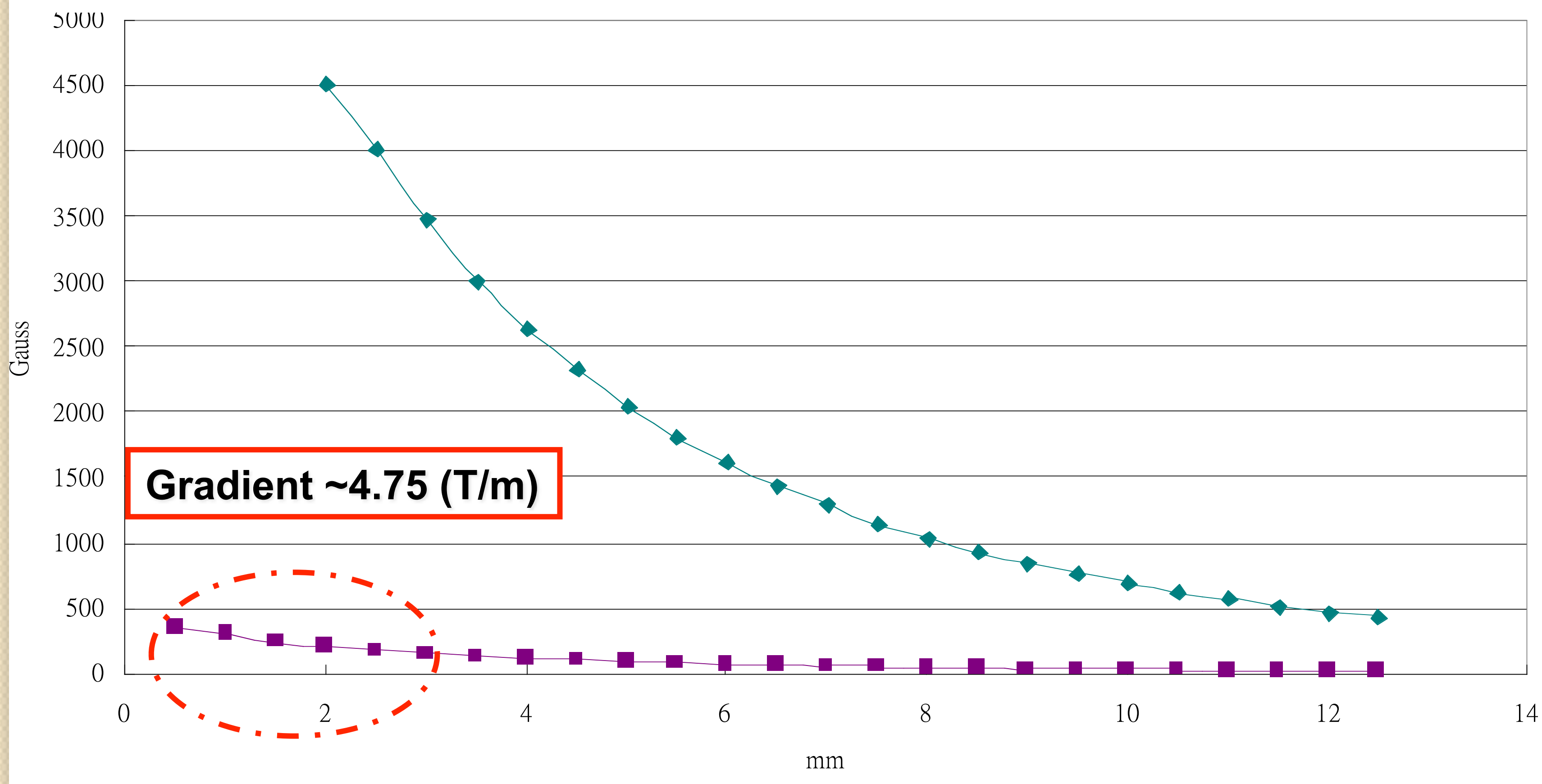
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Gauss
Gradient ~4.75 (T/m)
0
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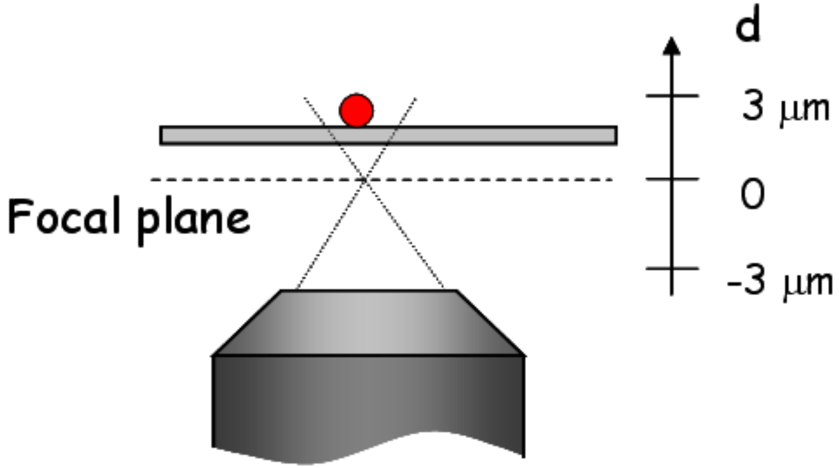
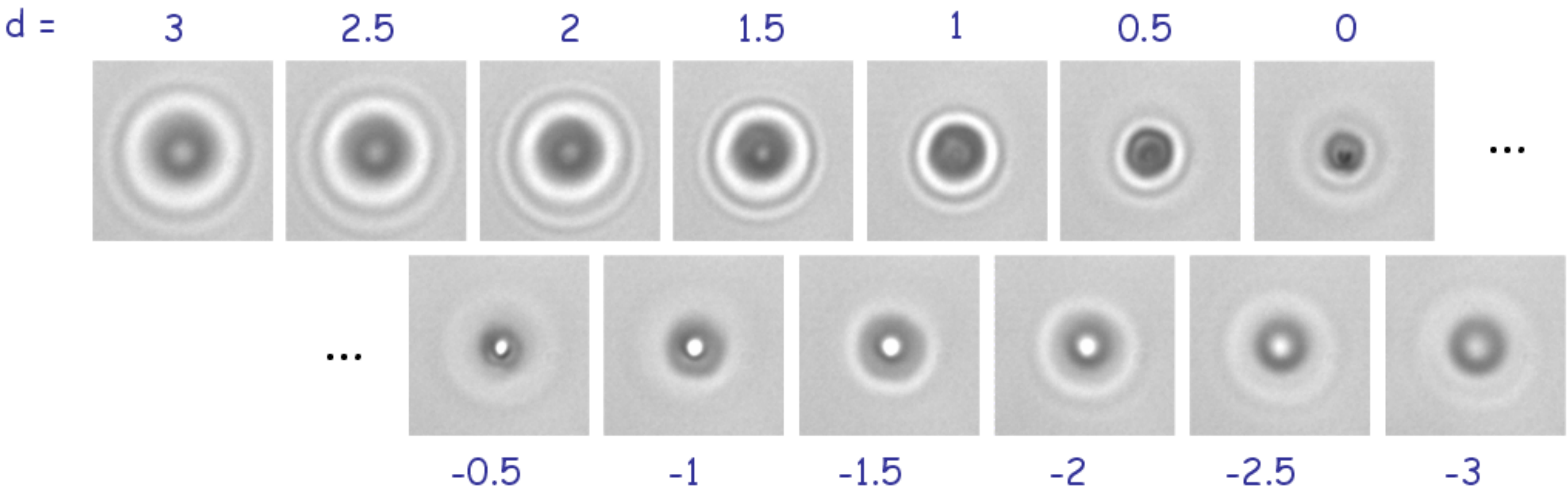
8
10
12
14
mm

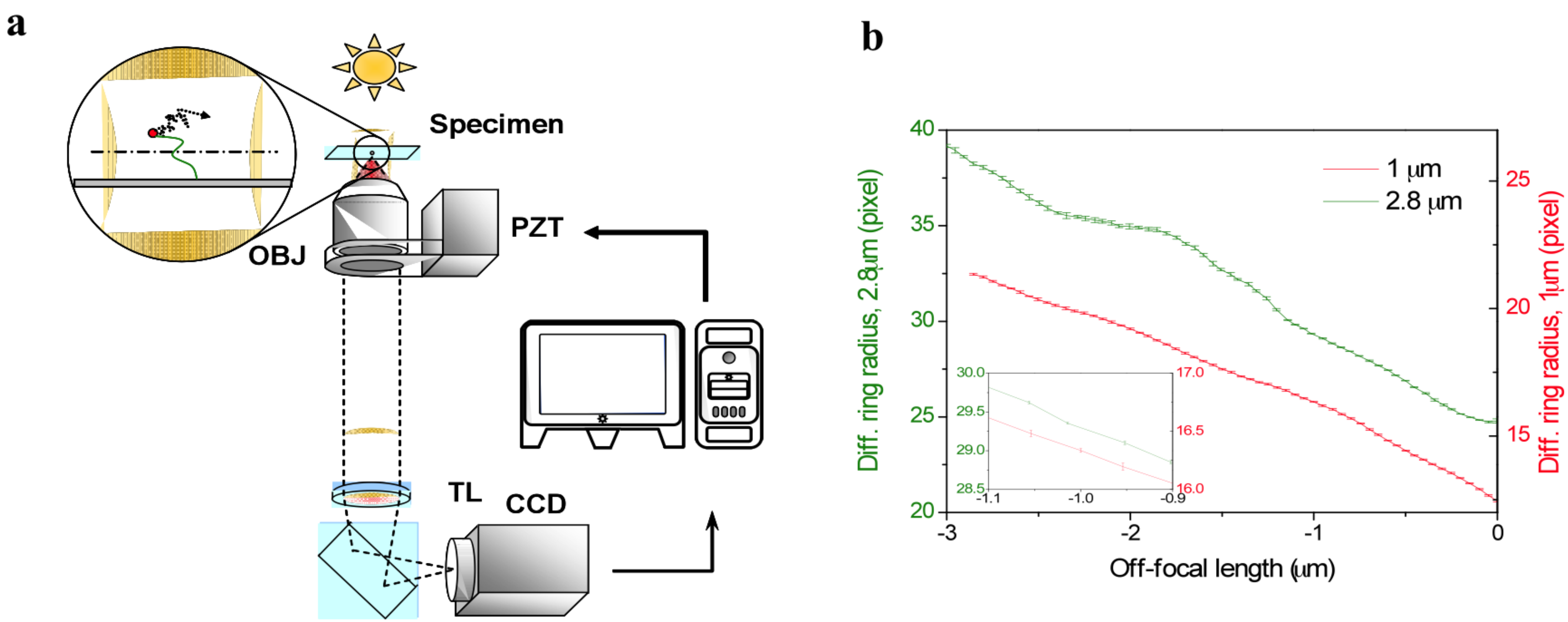
Single particle tracking system



The diffraction rings determines the z position of the bead.



Single particle tracking system – Scheme and calibration curve



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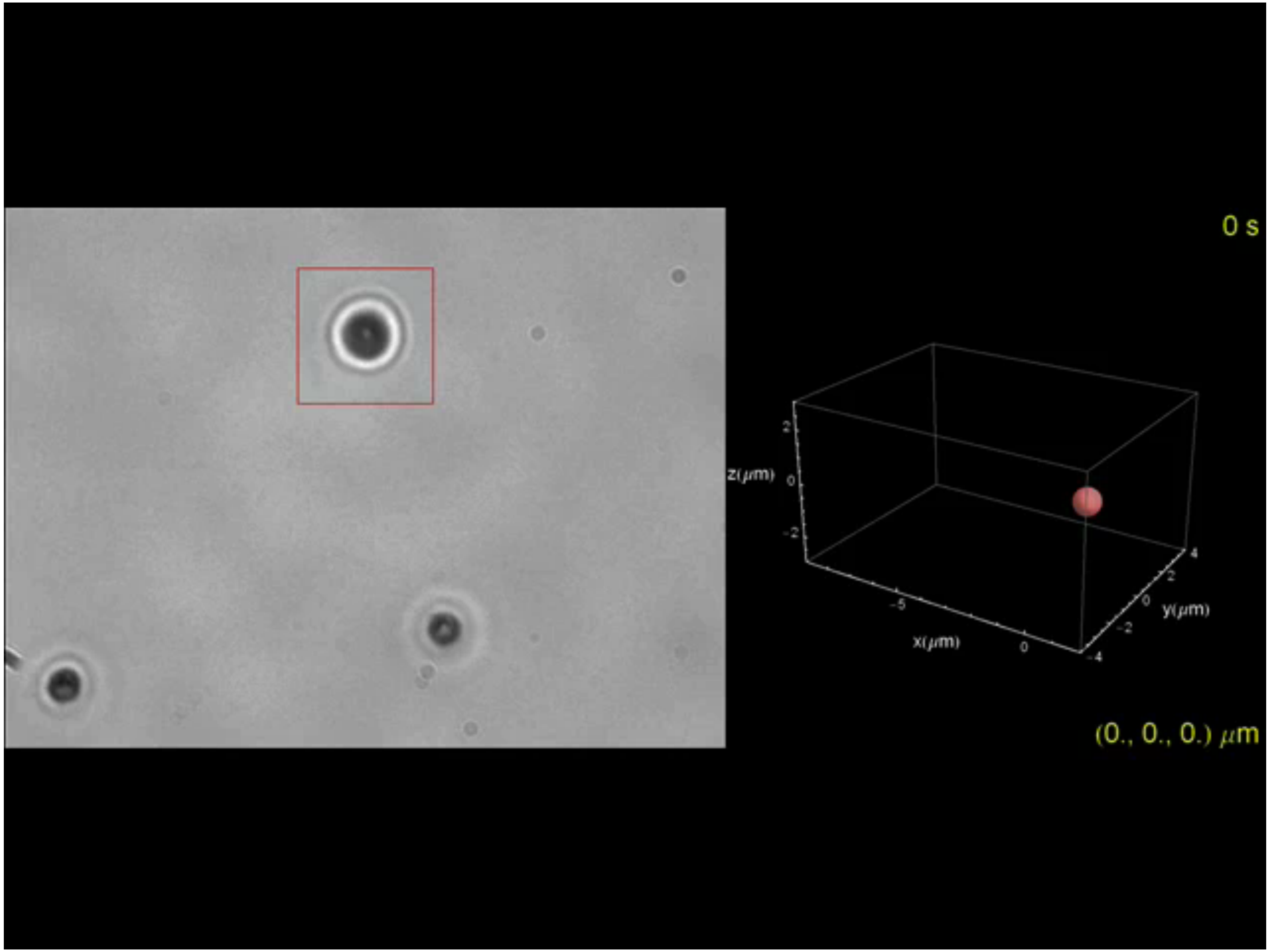
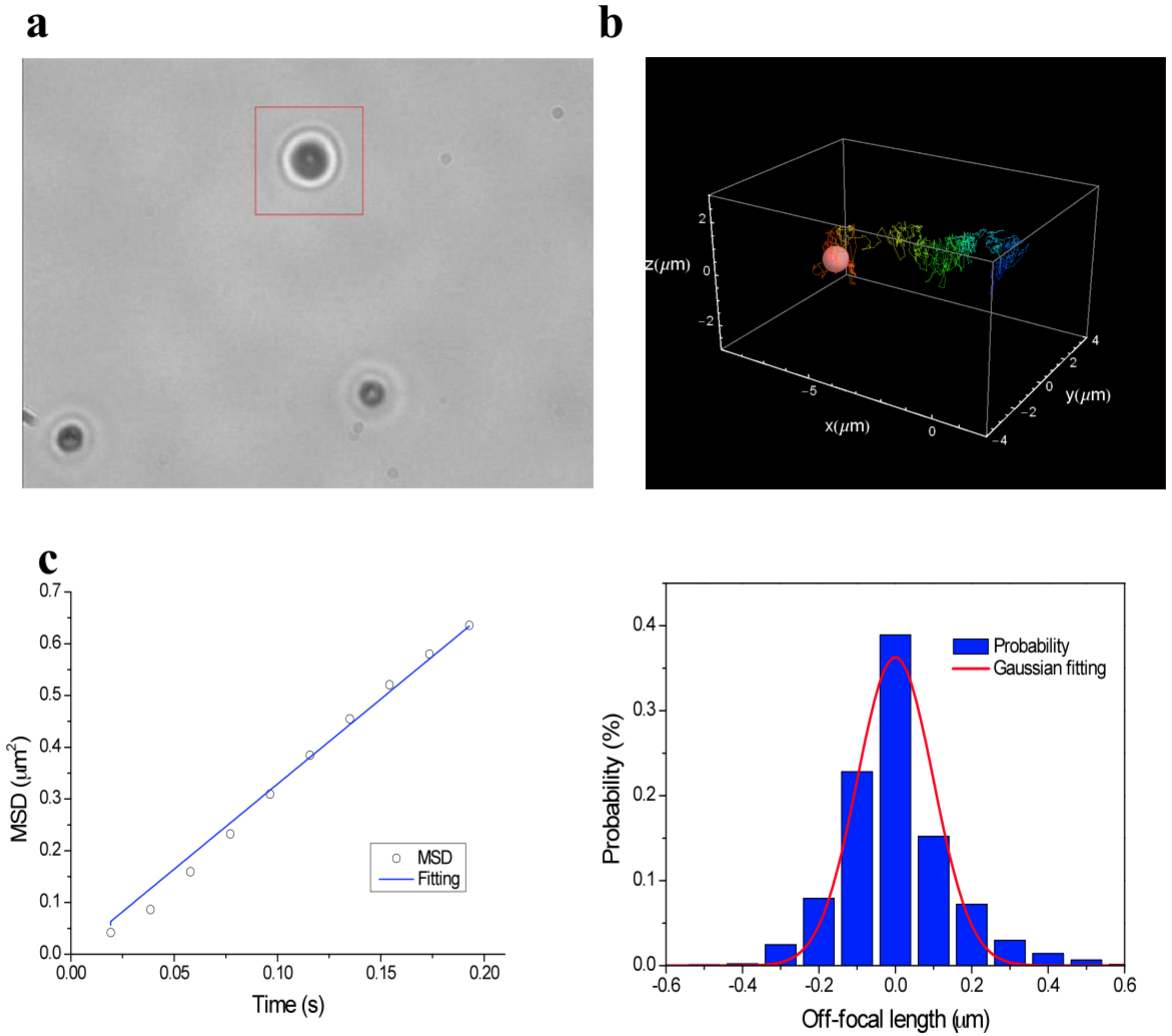
Free bead with tracking-I



Nanobioscience Lab

Free bead with tracking-II



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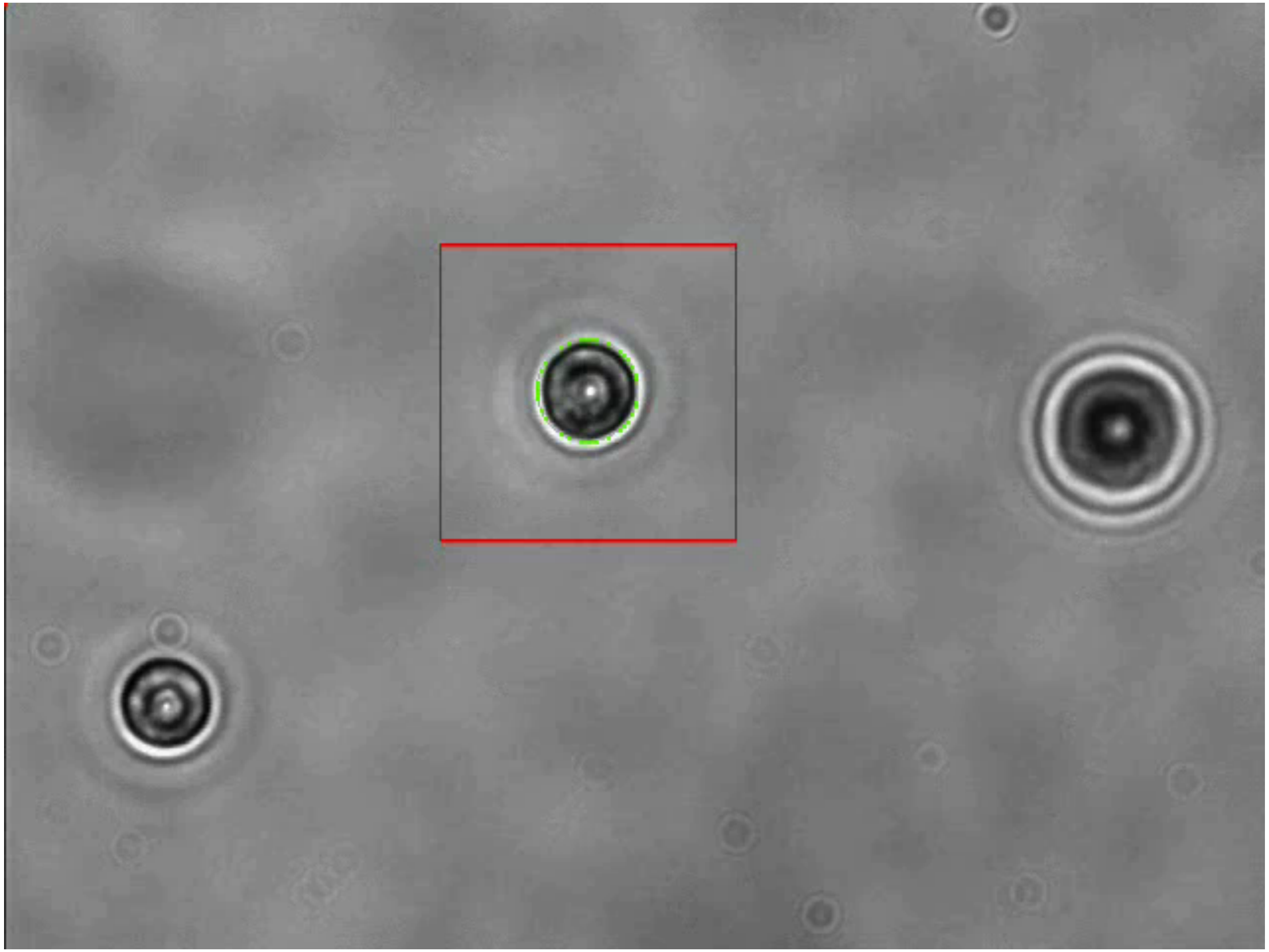
Bead-DNA tethering



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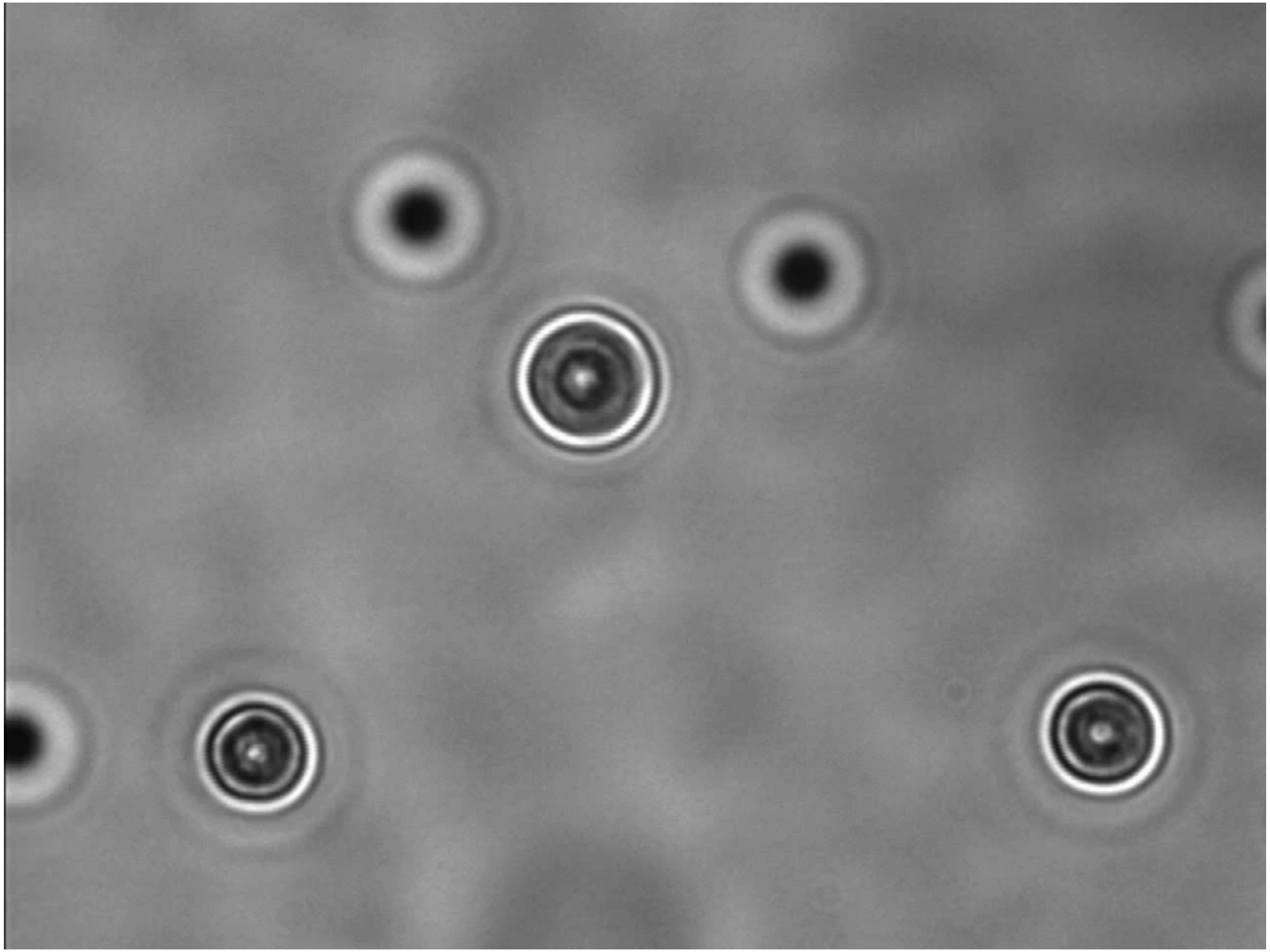
Multiple bead-DNA tethering



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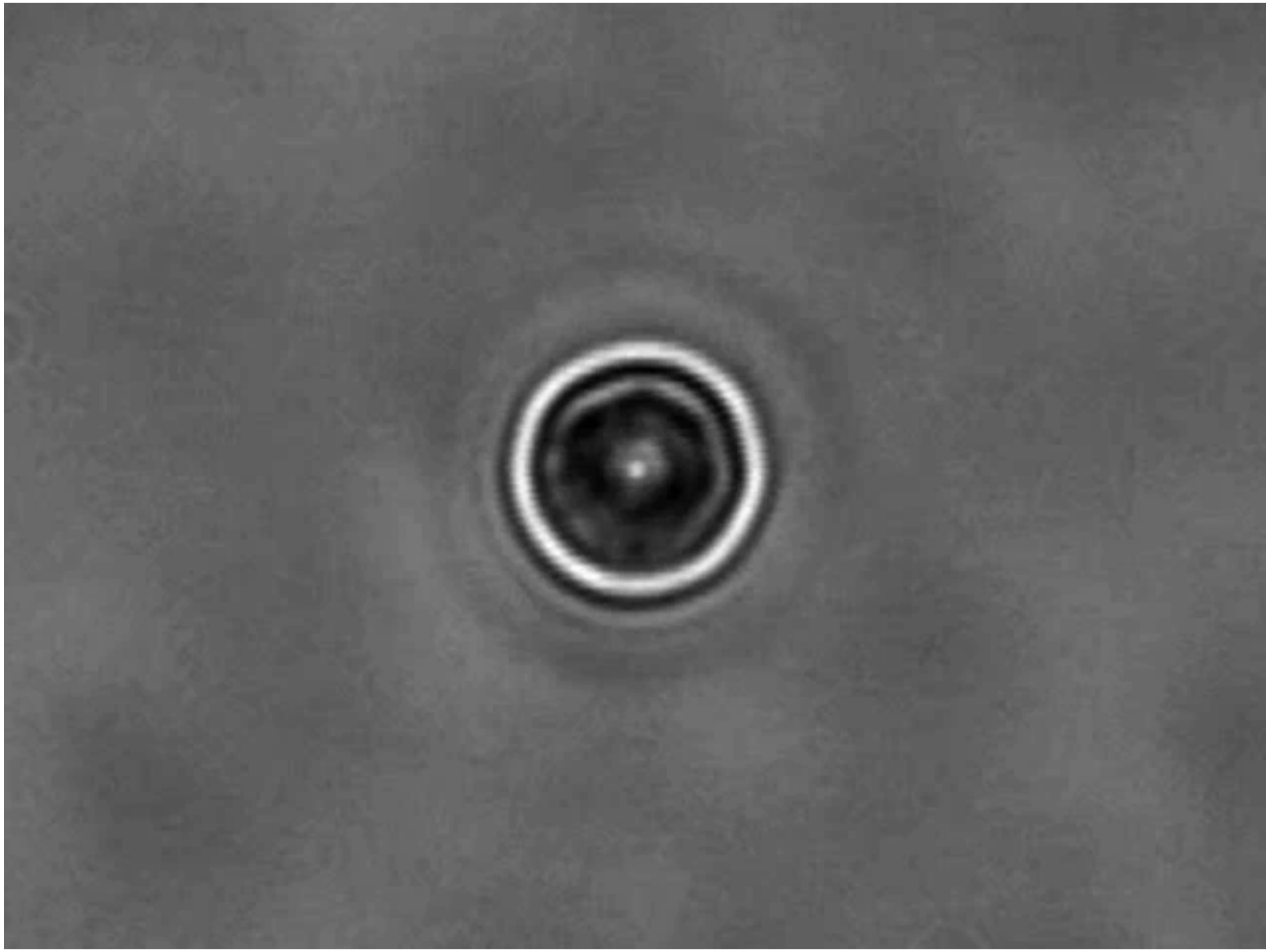
Bead-DNA rotation



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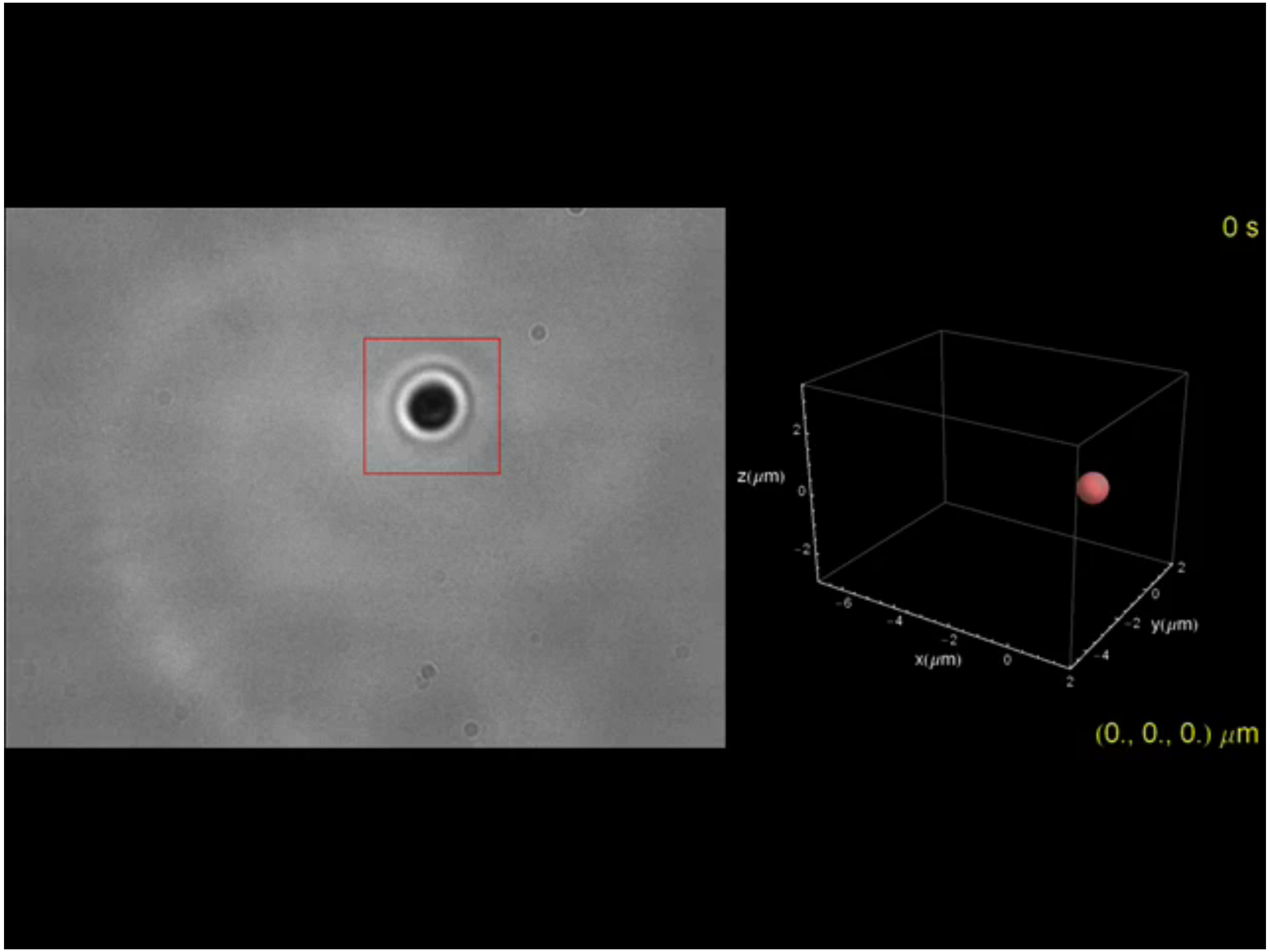
Bead-DNA tethering with drift-I



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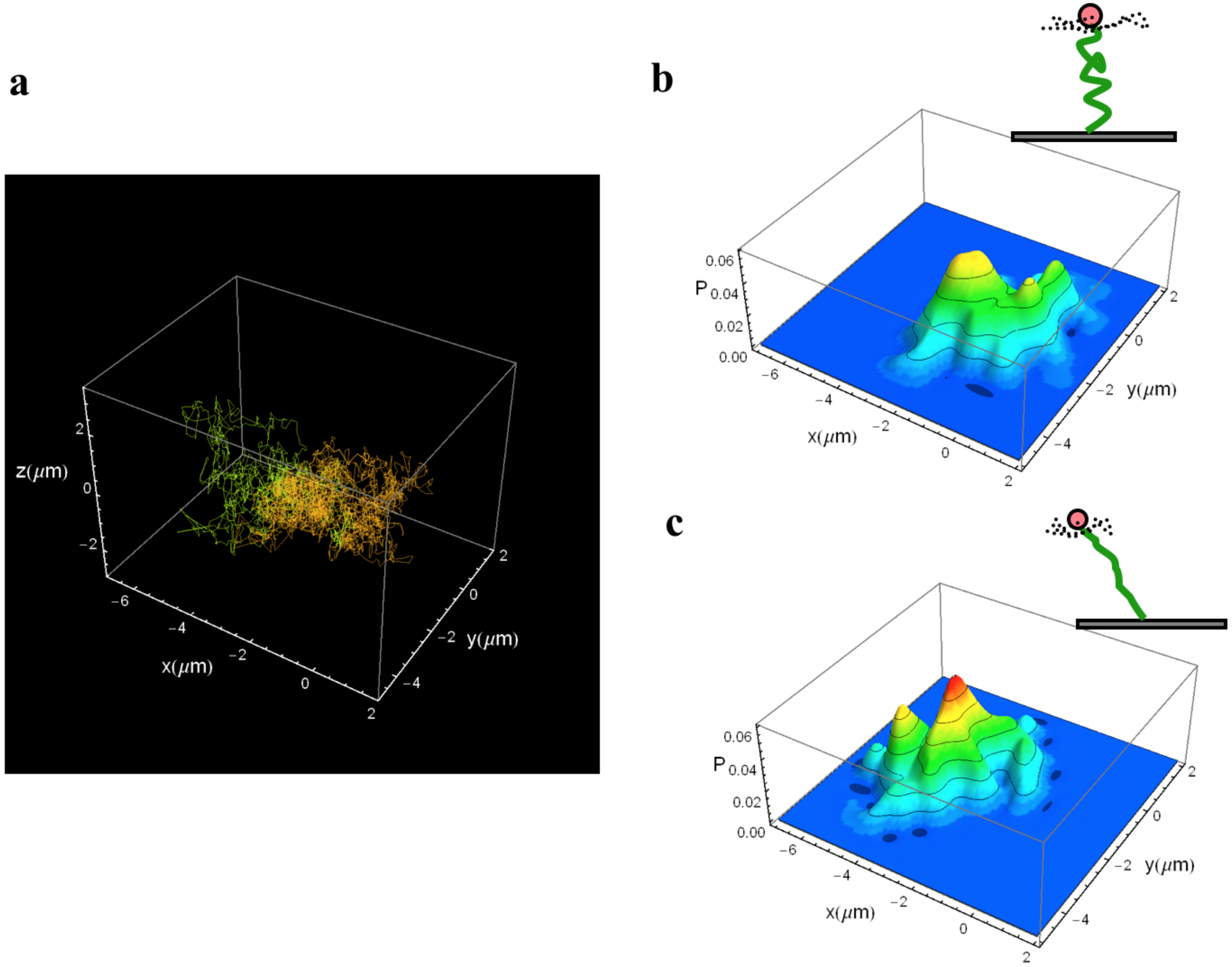
Bead-DNA tethering with drift-II



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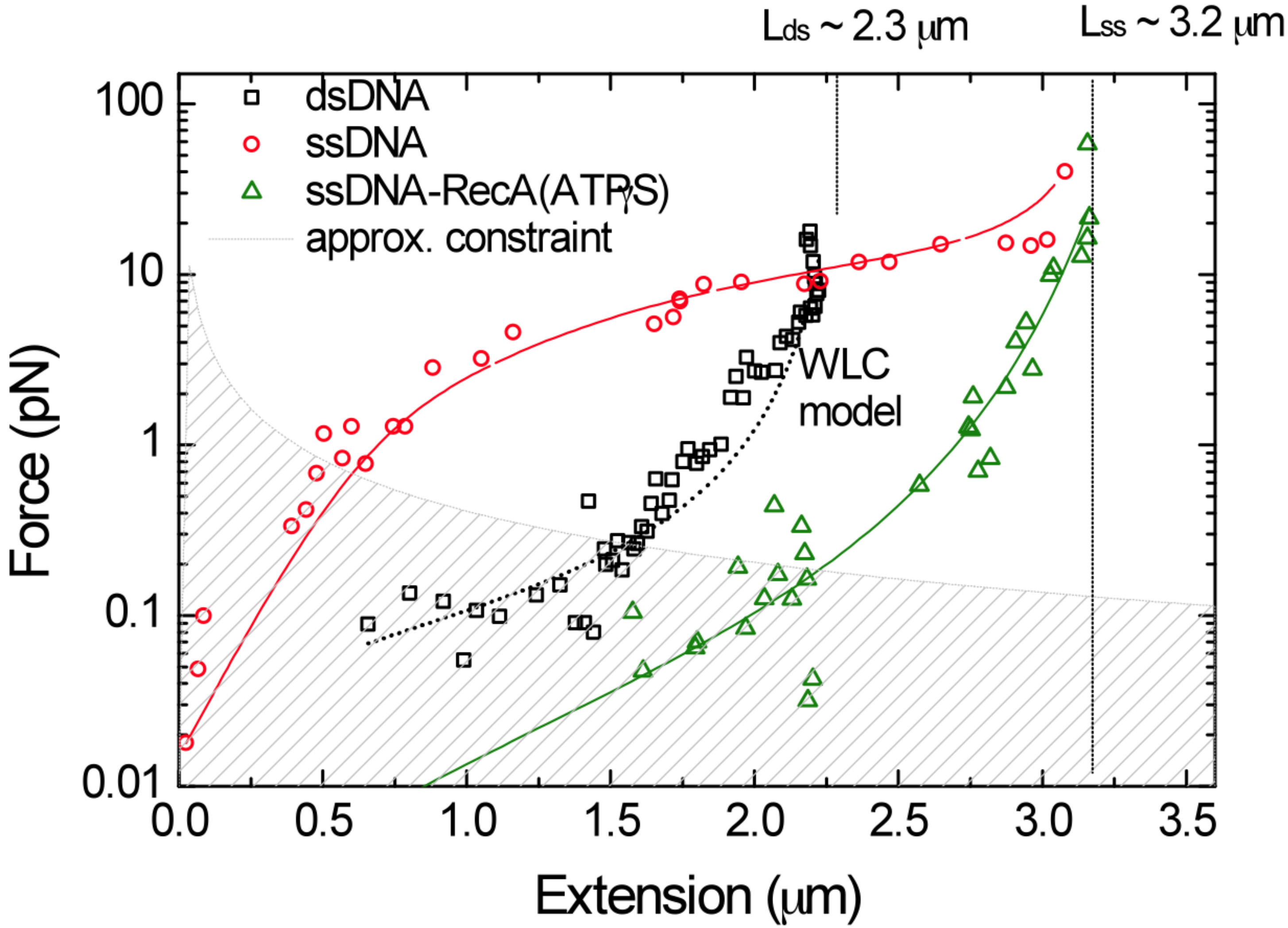
The force-extension curves



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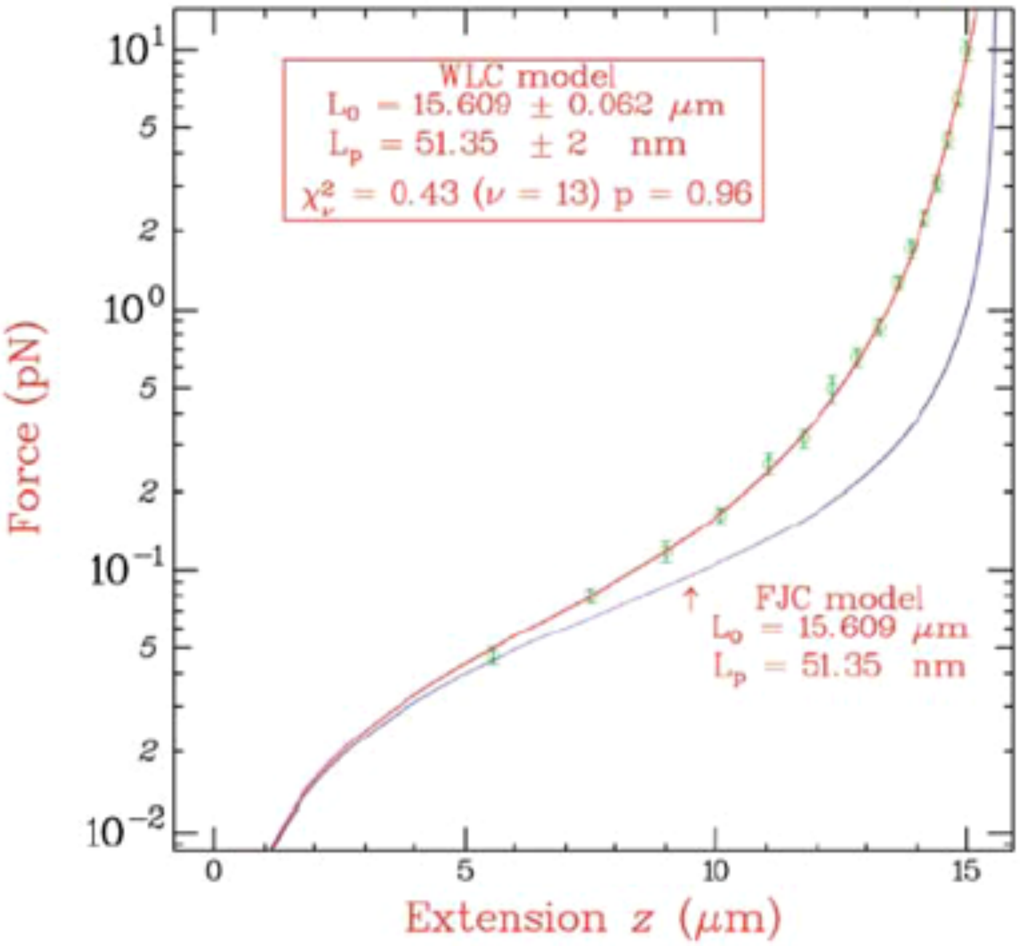
Force-extension curve of λ -DNA



Wormlike Chain Model:

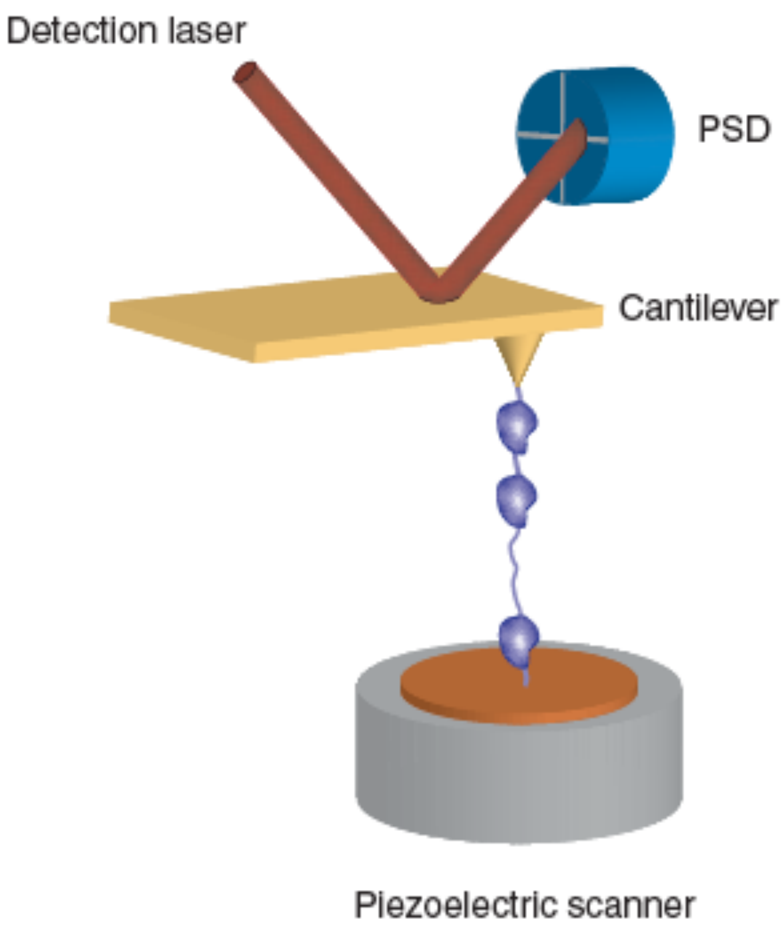
$$\frac{fA}{k_B T} = \frac{z}{L} + \frac{1}{4(1 - z/L)^2} - \frac{1}{4}$$

A: persistence length

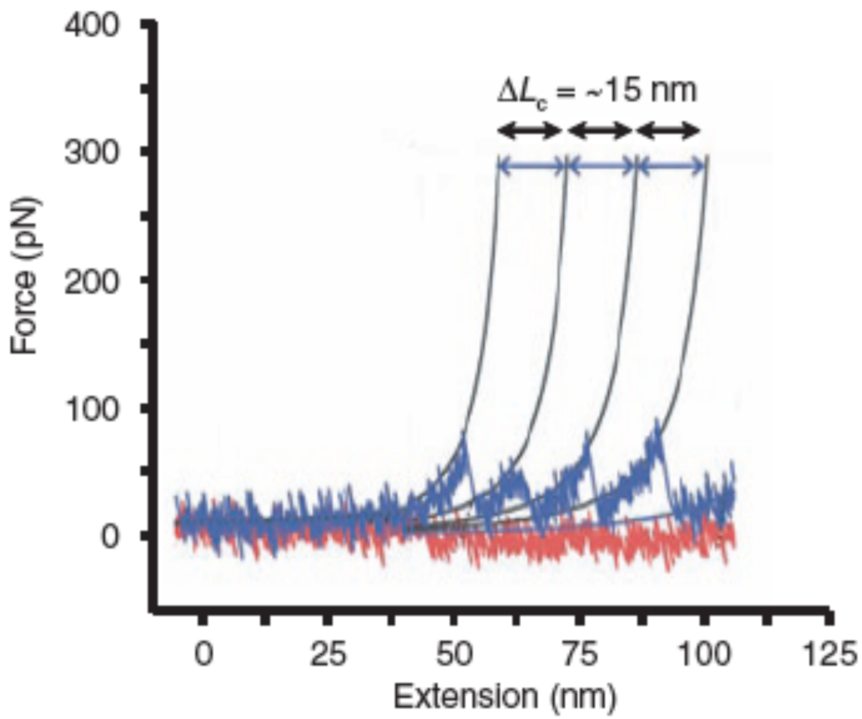
L: contour length

T.R. Strick et al., C. R. Physique 3 (2002) 595–618

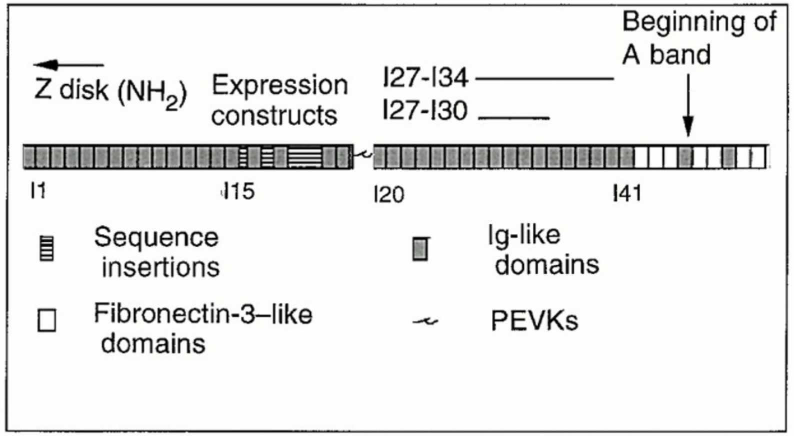
Atomic force microscopy



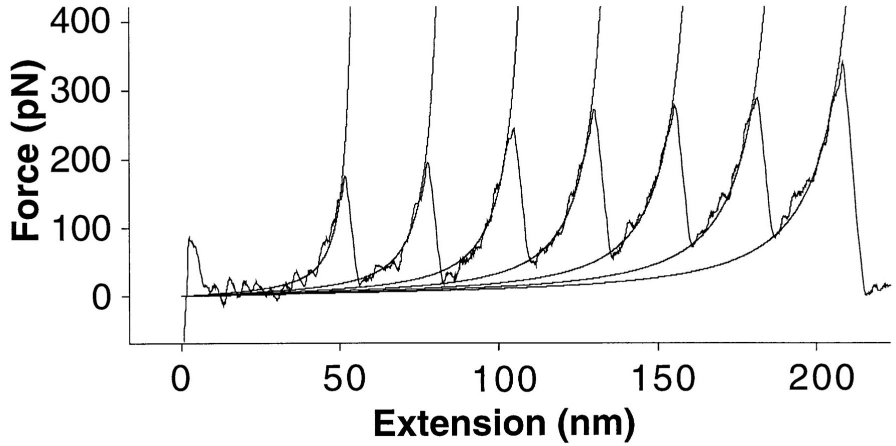
Wormlike Chain Model:

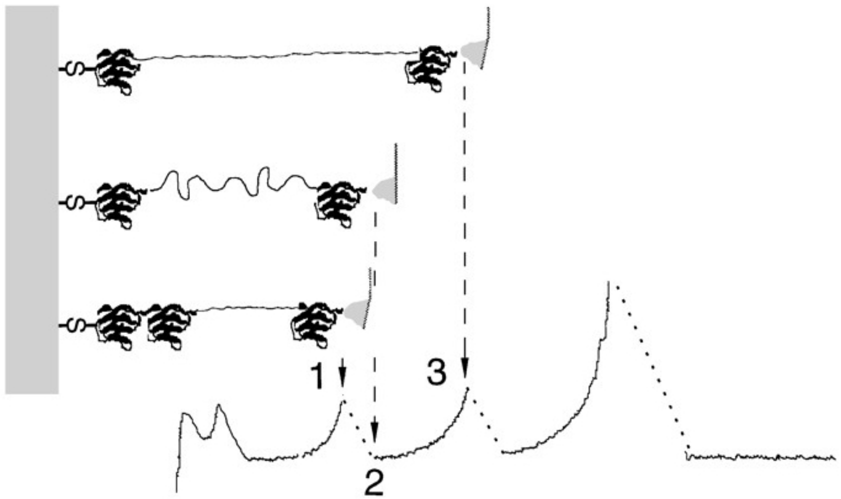


Protein elasticity: unfolding titin



Fitting to wormlike Chain Model:





Rief et al., *Science* (1997) 276 :1109-1112

Table 1 | Comparison of single-molecule force spectroscopy techniques

	Optical tweezers	Magnetic (electromagnetic) tweezers	AFM
Spatial resolution (nm)	0.1–2	5–10 (2–10)	0.5–1
Temporal resolution (s)	10^{-4}	10^{-1}–10^{-2} (10^{-4})	10^{-3}
Stiffness (pN nm^{-1})	0.005–1	10^{-3}–10^{-6} (10^{-4})	10–10^{5}
Force range (pN)	0.1–100	10^{-3}–10^{2} (0.01–10^{4})	10–10^{4}
Displacement range (nm)	0.1–10^{5}	5 –10^{4} (5–10^{5})	0.5–10^{4}
Probe size (μm)	0.25–5	0.5–5	100–250
Typical applications	3D manipulation Tethered assay Interaction assay	Tethered assay DNA topology (3D manipulation)	High-force pulling and interaction assays
Features	Low-noise and low-drift dumbbell geometry	Force clamp Bead rotation Specific interactions	High-resolution imaging
Limitations	Photodamage Sample heating Nonspecific	No manipulation (Force hysteresis)	Large high-stiffness probe Large minimal force Nonspecific

KC Neuman & A Nagy. NATURE METHODS | VOL.5 NO.6 | JUNE 2008 | 491-505.

Further readings:

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2. Svoboda K., Block S. M. 1994. "Biological applications of optical forces." Annu Rev Biophys Biomol Struct 23:247-85.
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7. Engel, A. Biological applications of scanning probe microscopes. Annu. Rev. Biophys. Biophys. Chem. 20, 79–108 (1991).
8. Shao, Z., Yang, J. & Somlyo, A.P. Biological atomic force microscopy: frommicrons to nanometers and beyond. Annu. Rev. Cell Dev. Biol. 11, 241–265 (1995).