



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 μ m.

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

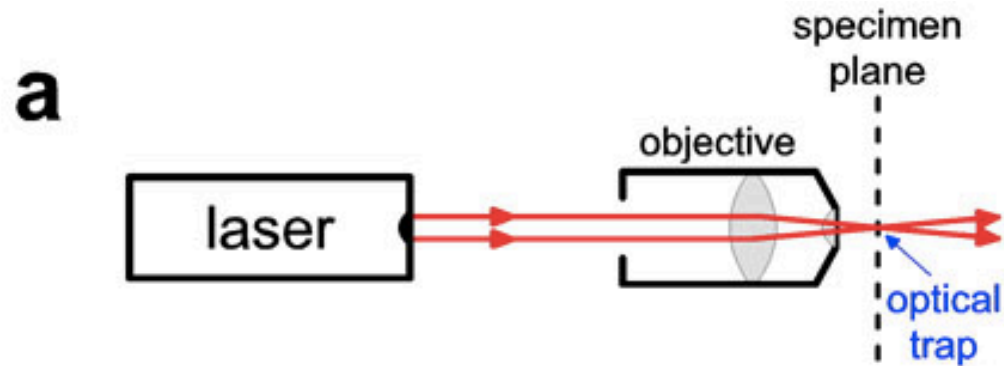


Claude Cohen-Tannoudji

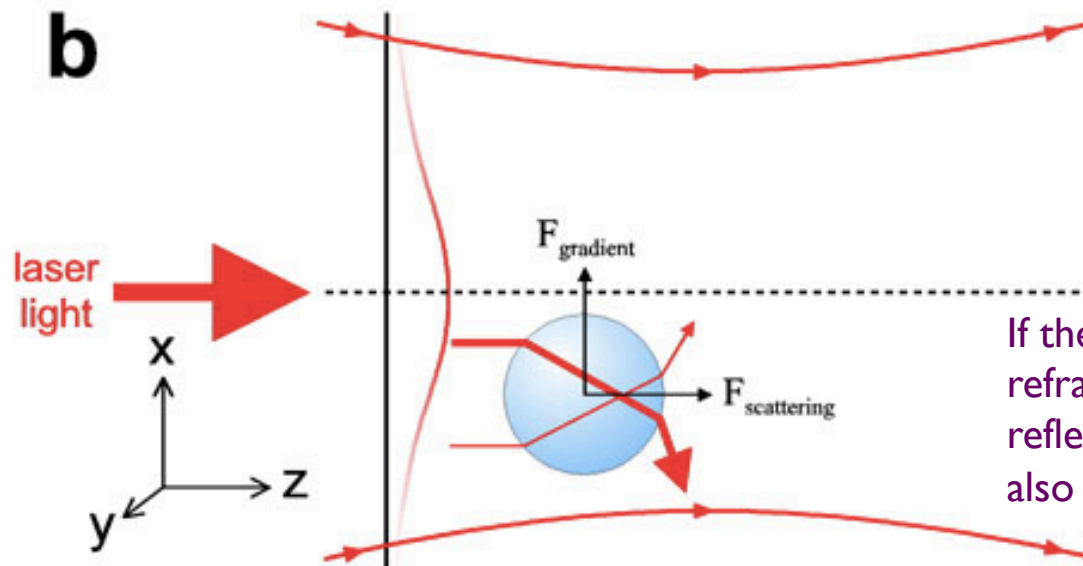


William D. Phillips

Optical Tweezers—principles



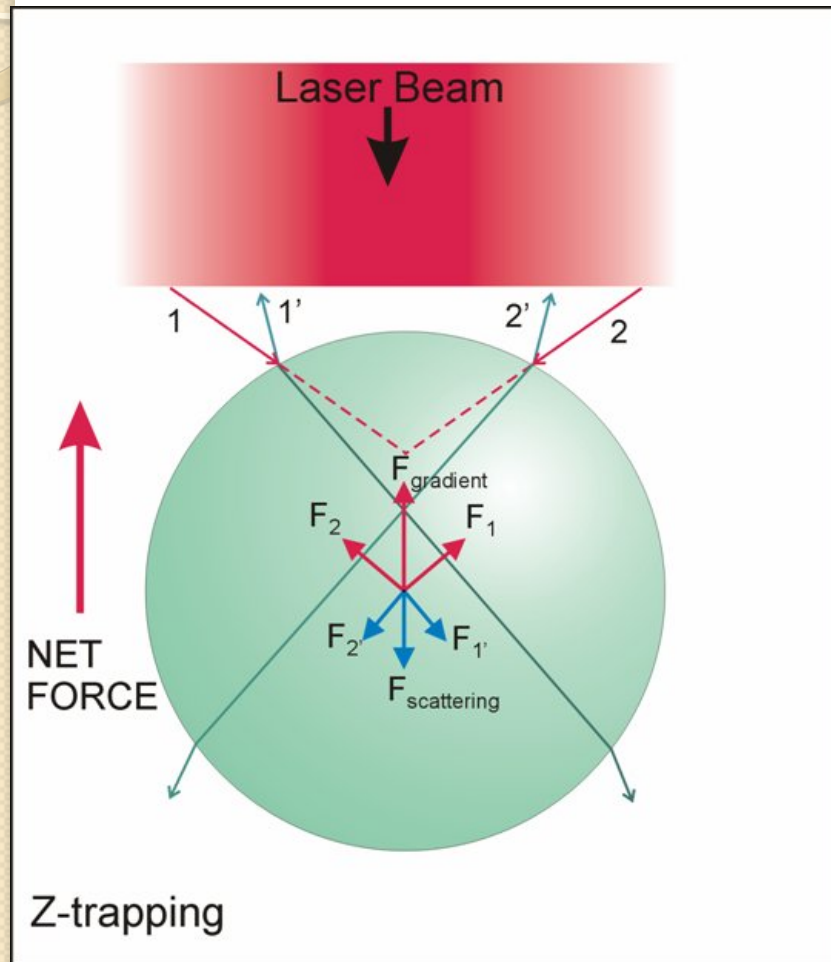
The $\mathbf{F}_{\text{gradient}}$ is a restoring force that pulls the bead into the center.



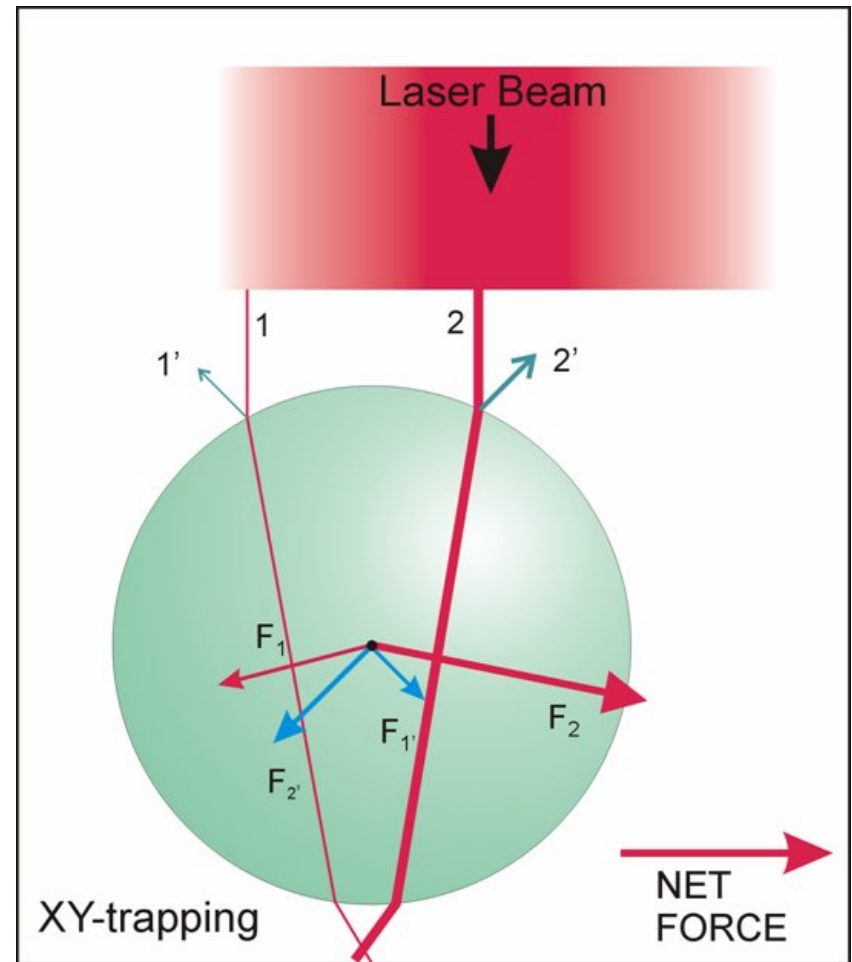
If the contribution to $\mathbf{F}_{\text{scattering}}$ of the refracted rays is larger than that of the reflected rays then a restoring force is also created along the z-axis.

Ray optics of momentum change

Lateral Trapping

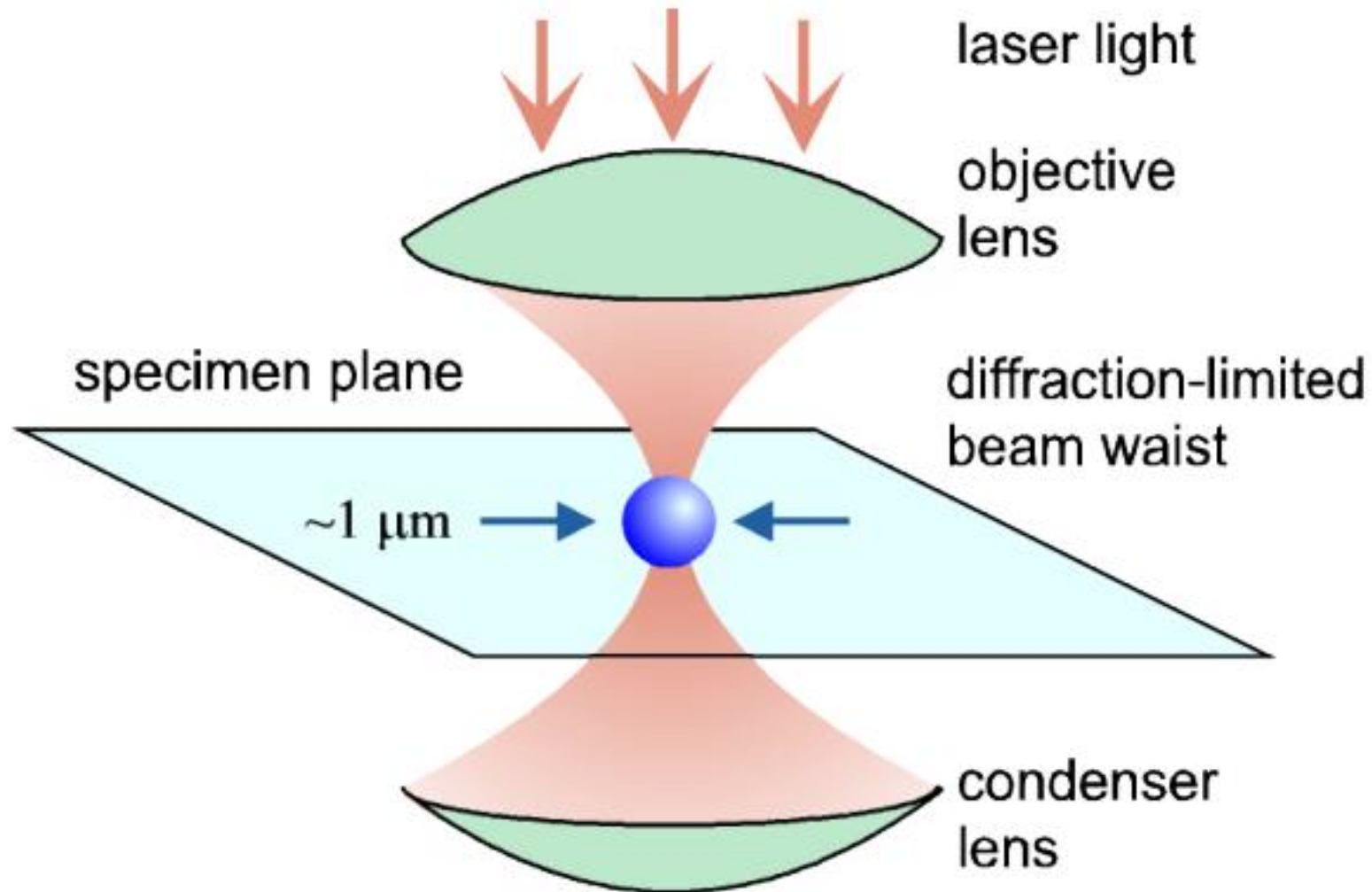


Axial Trapping



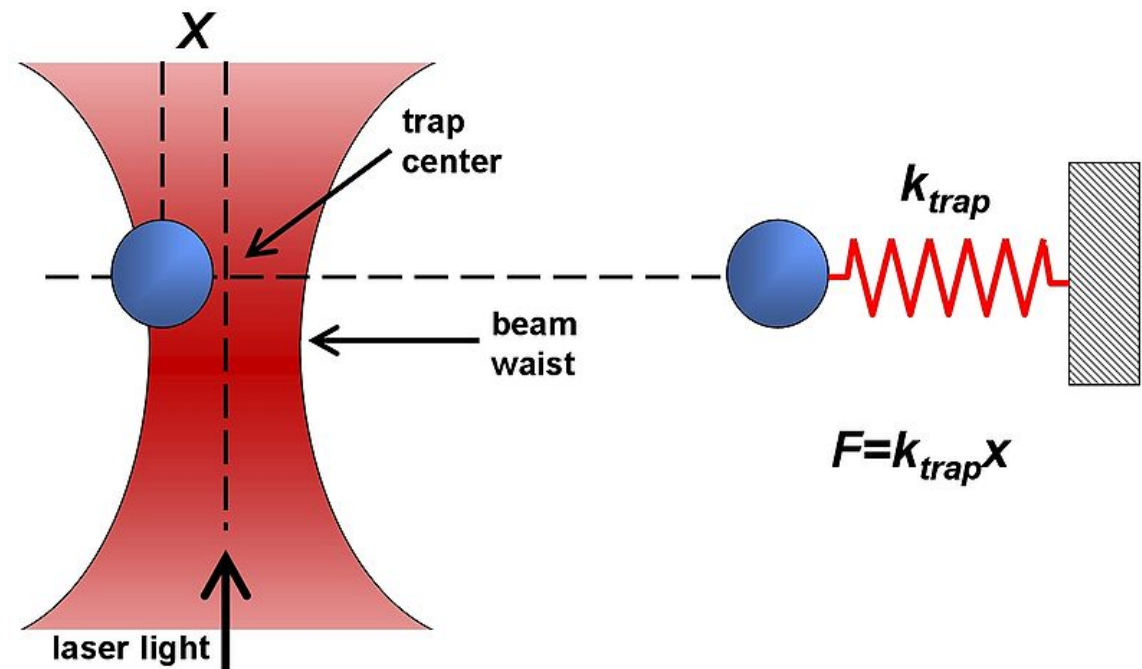
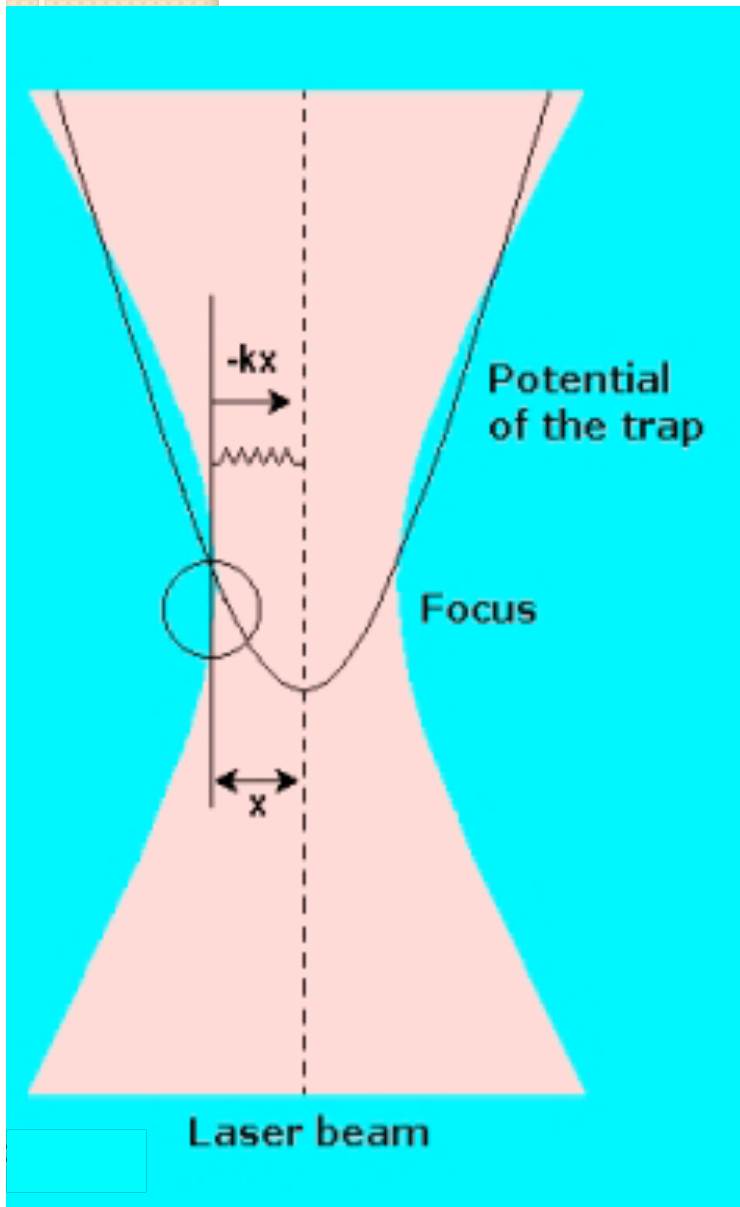
Optical tweezers

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





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:

$$U = -\mathbf{p} \cdot \mathbf{E} = -\alpha \nabla E^2$$

Dielectrophoretic force:

$$\mathbf{F} = -\nabla U \sim E \nabla E$$

$$F = 2\pi a^3 \epsilon_m \operatorname{Re} \left(\frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \right) \nabla(E^2)$$

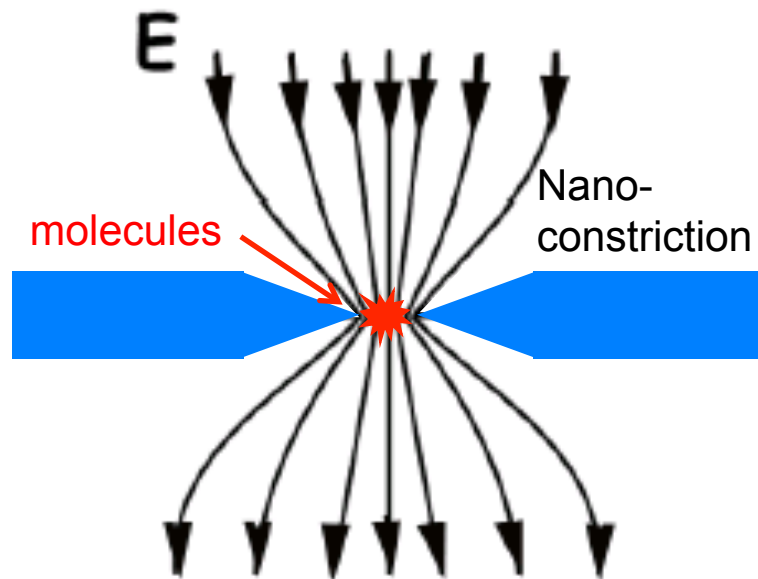
Sphere of radius a ,
 $\epsilon_m = 80$ for water

Clausius-Mossotti factor



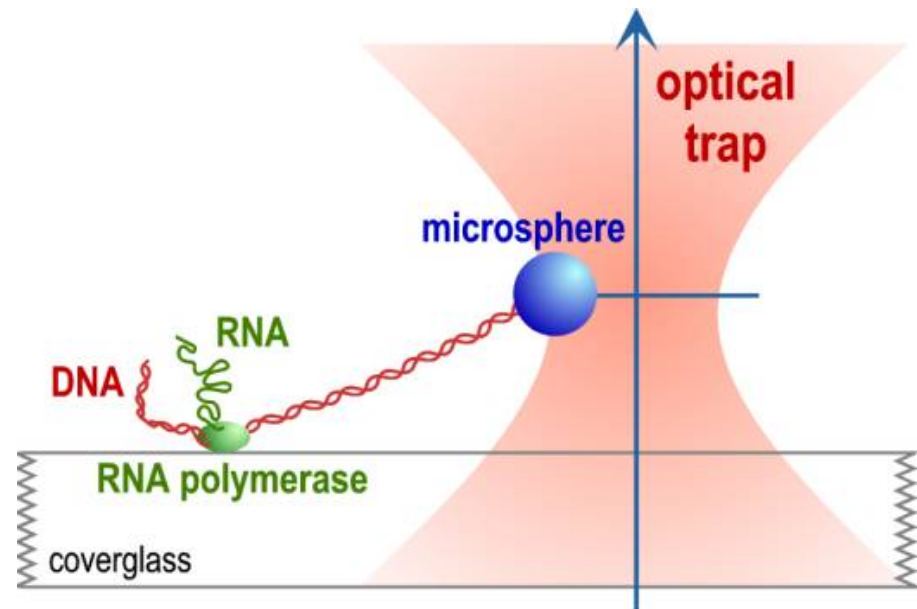
EDEP Molecular Trap vs. Optical Tweezers

EDEP molecular trap



Electric field focused
at the constriction

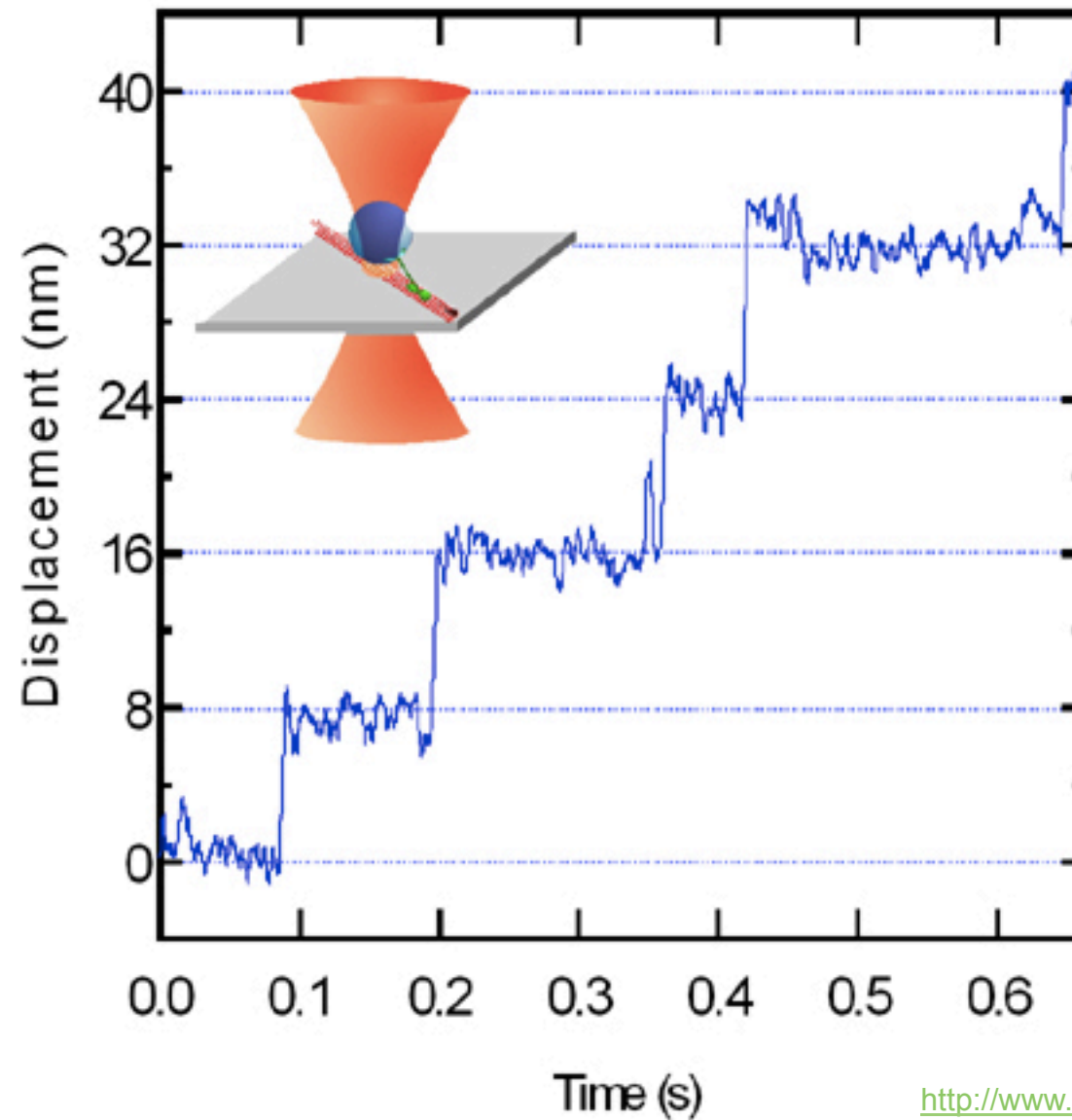
Optical trap



Electric field focused
at the focal point



Measurement of the 8-nm steps of kinesin



The 'dumbbell' experiment

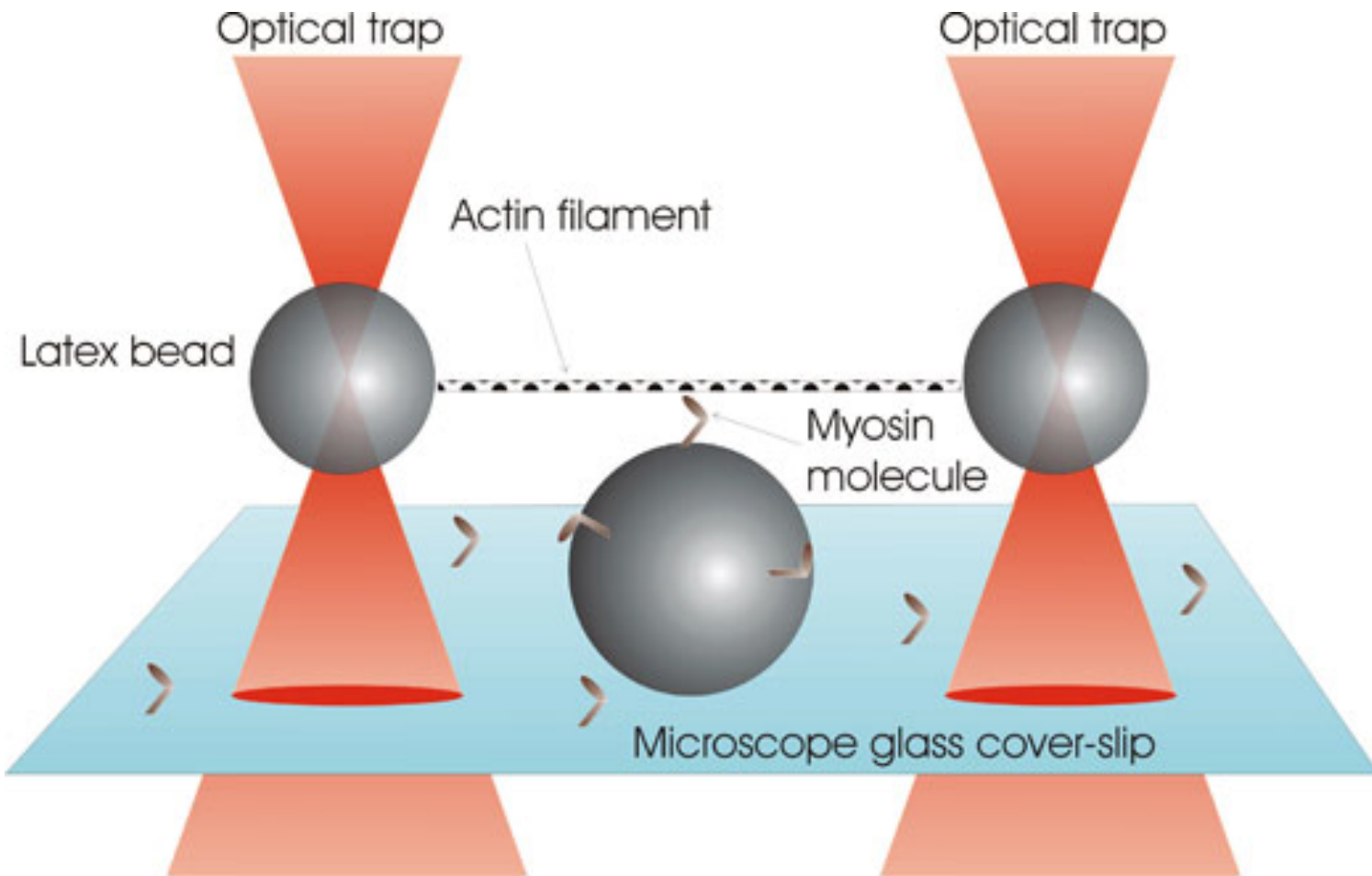
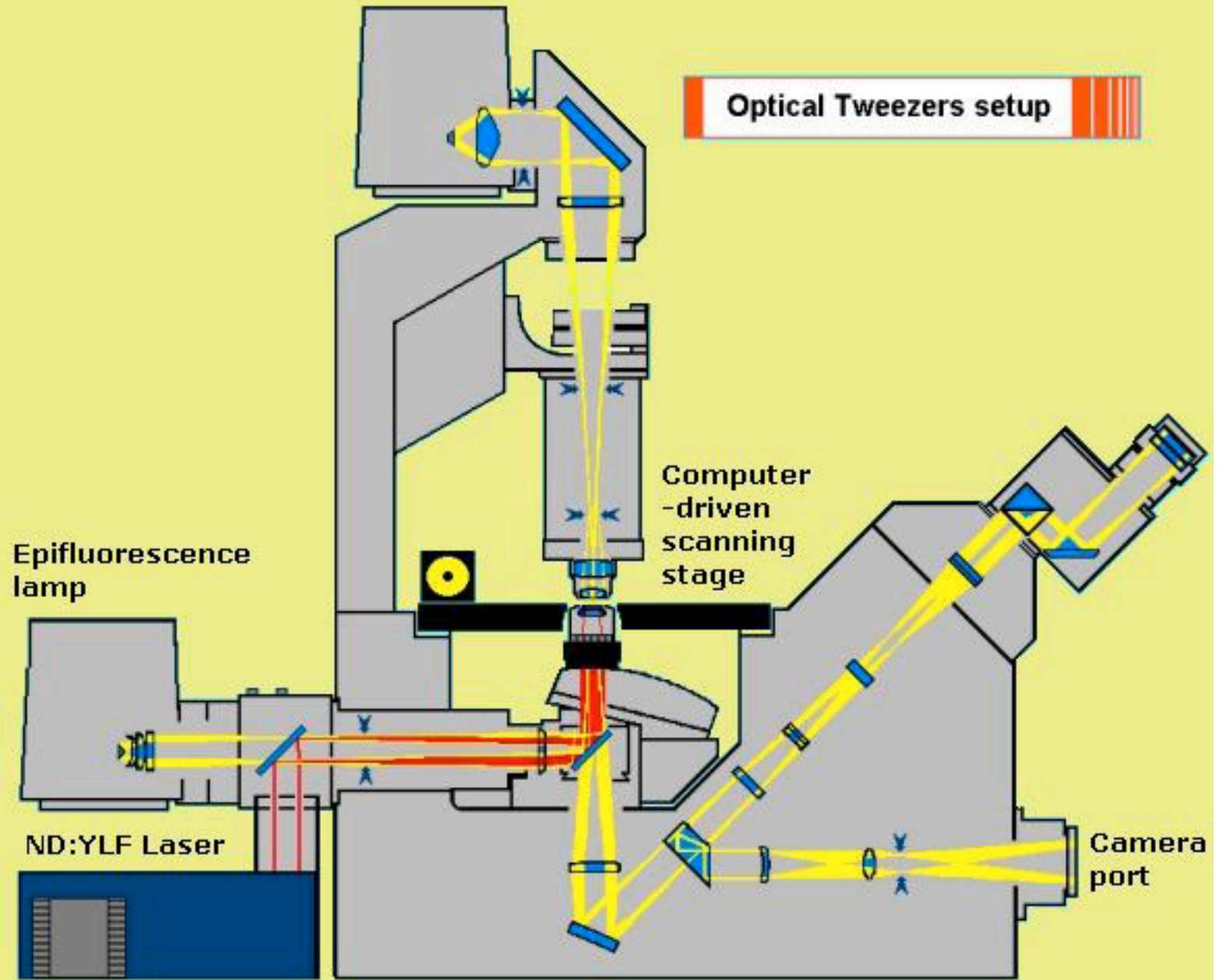
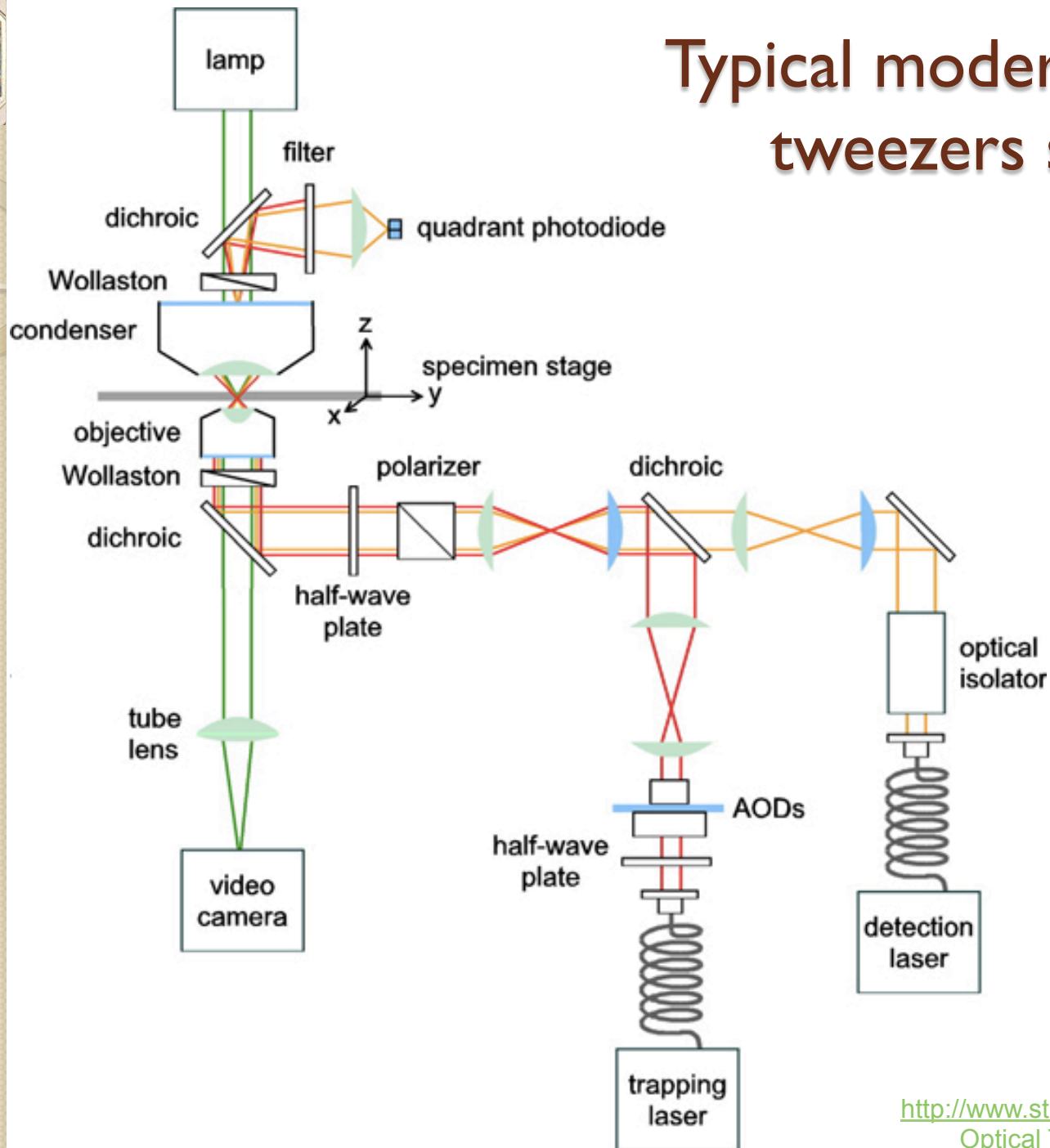


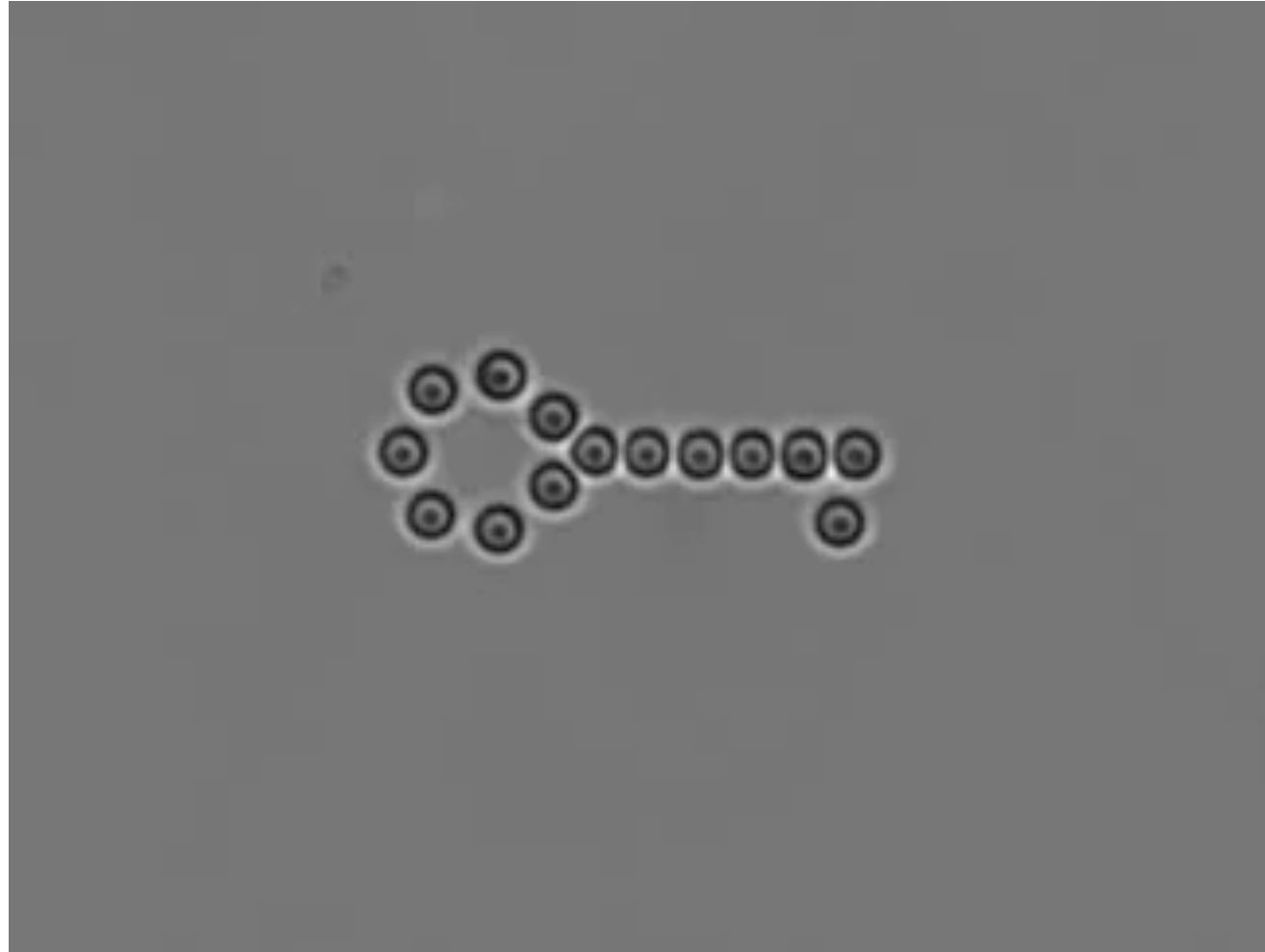
Image courtesy of Alexandre Lewalle



Typical modern optical tweezers setup

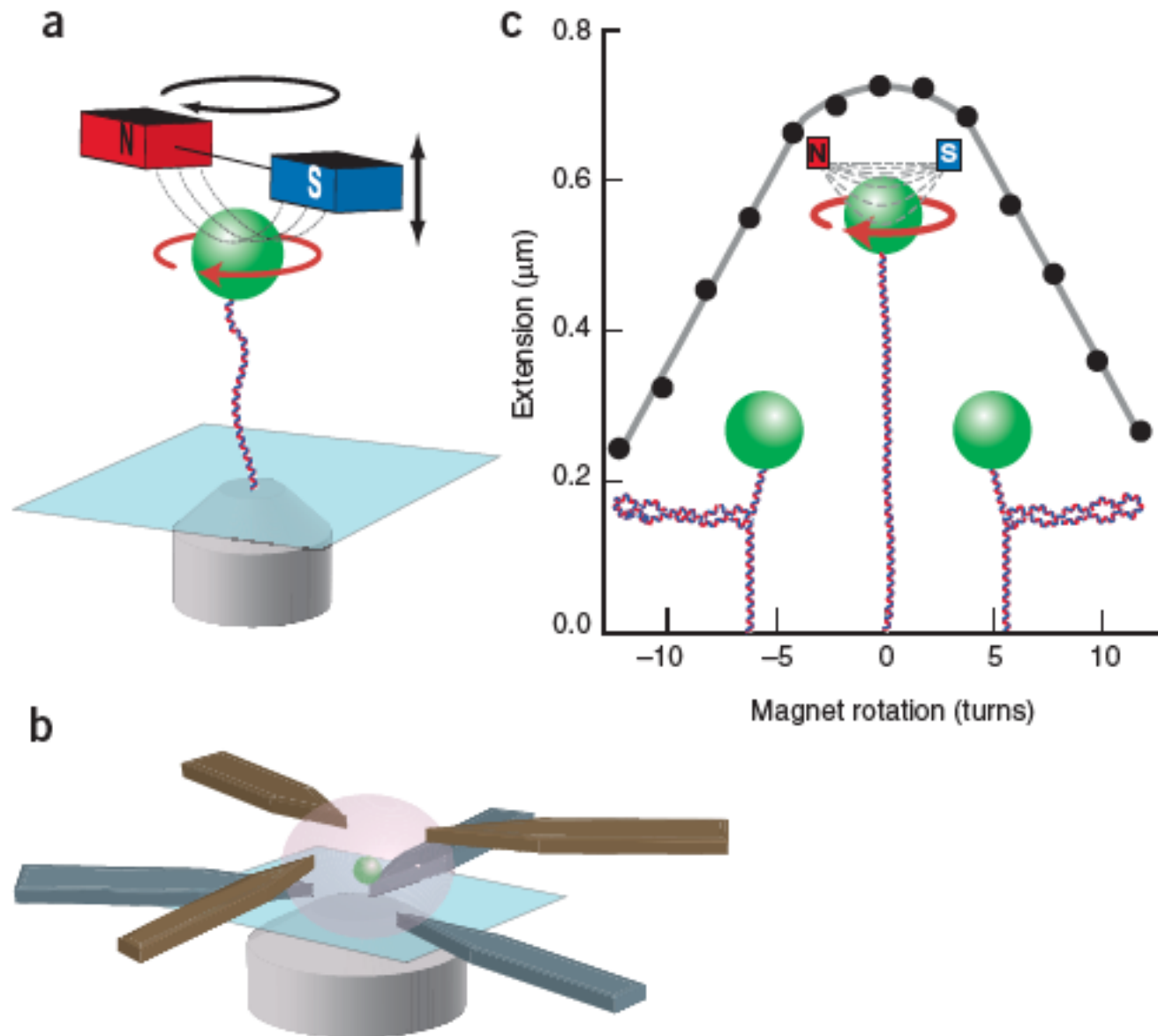


Optical Tweezers in Action



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

Magnetic Tweezers for Single Molecule Manipulation



Theory of Magnetic Tweezers

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

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

Assumption:

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

4.5- μm diameter bead $\rightarrow$

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

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

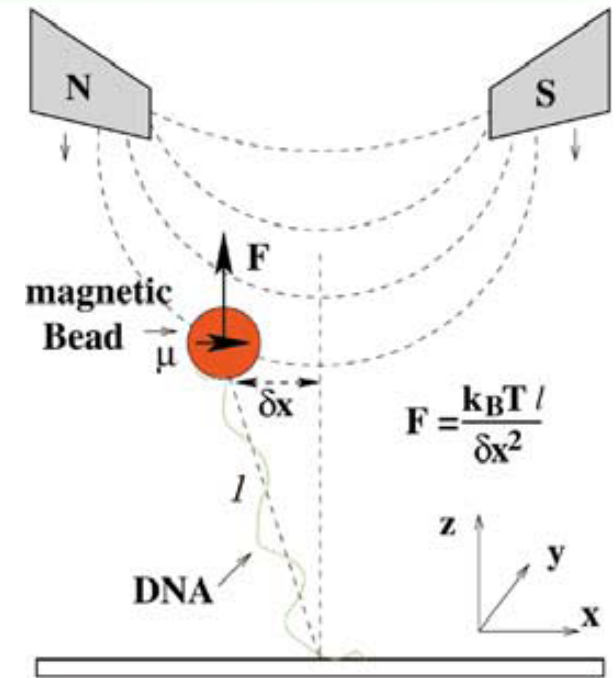
$$\rightarrow F_{\text{magnetic}} \propto \frac{\partial B}{\partial z}$$

As the field gradient $\sim 100 \text{ T/m}$

4.5- μm diameter bead $\rightarrow F_{\text{magnetic}} : > 60 \text{ pN}$

2.8- μm diameter bead $\rightarrow F_{\text{magnetic}} : \sim 14 \text{ pN}$

1.0- μm diameter bead $\rightarrow F_{\text{magnetic}} : \sim 2 \text{ pN}$



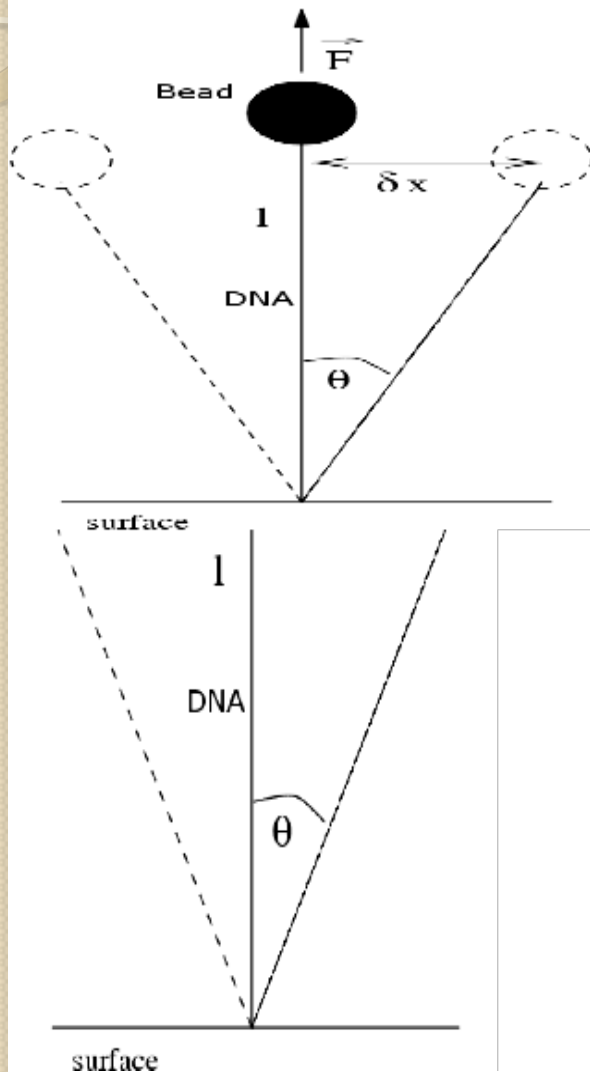


Force Calibration

- ❖ Calibration against flow field ($F=6\pi\eta r v$)
- ❖ 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:

$$\frac{1}{2} k_x \langle \delta x^2 \rangle = \frac{1}{2} k_B T$$

$$\frac{F}{l} \langle \delta x^2 \rangle = k_B T$$

$$F = \frac{k_B T l}{\langle \delta x^2 \rangle}$$

Magnetic Tweezers Setup

1. Magnetic tweezers configuration by Gauss meter
2. Magnetic force calibration by viscous drag force

Stokes' law

$$F_{\text{magnetic}} = 6\pi\eta r v_f$$

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

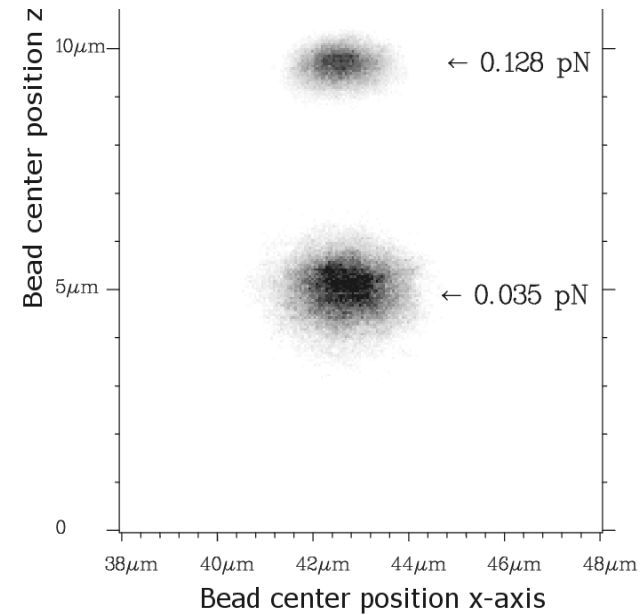
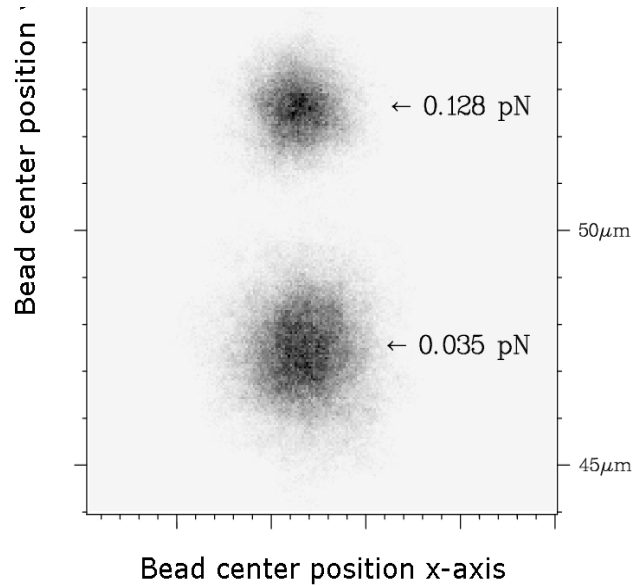
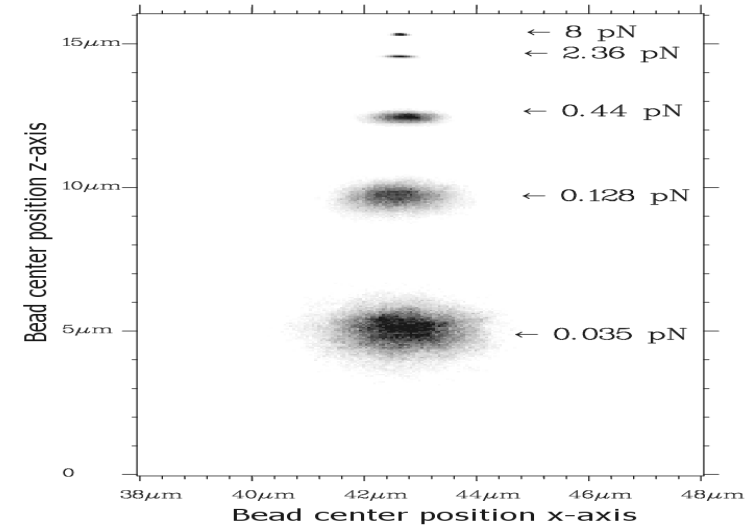
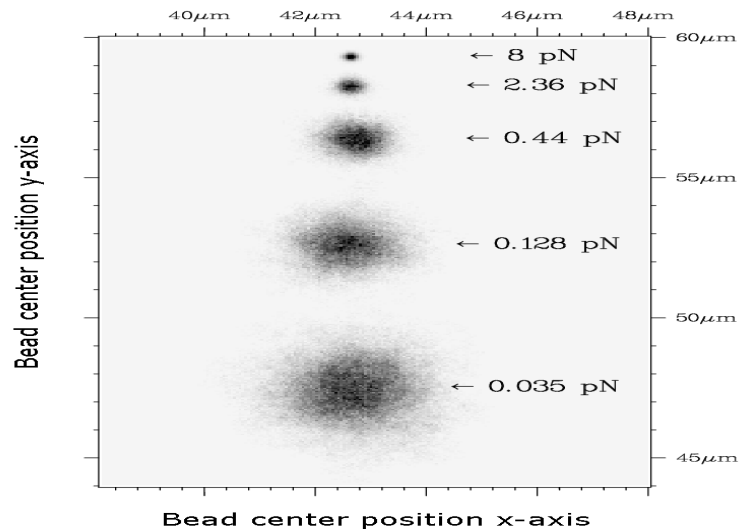
Equipartition theorem

$$\delta x^2 = \frac{K_b T \ell}{F}$$

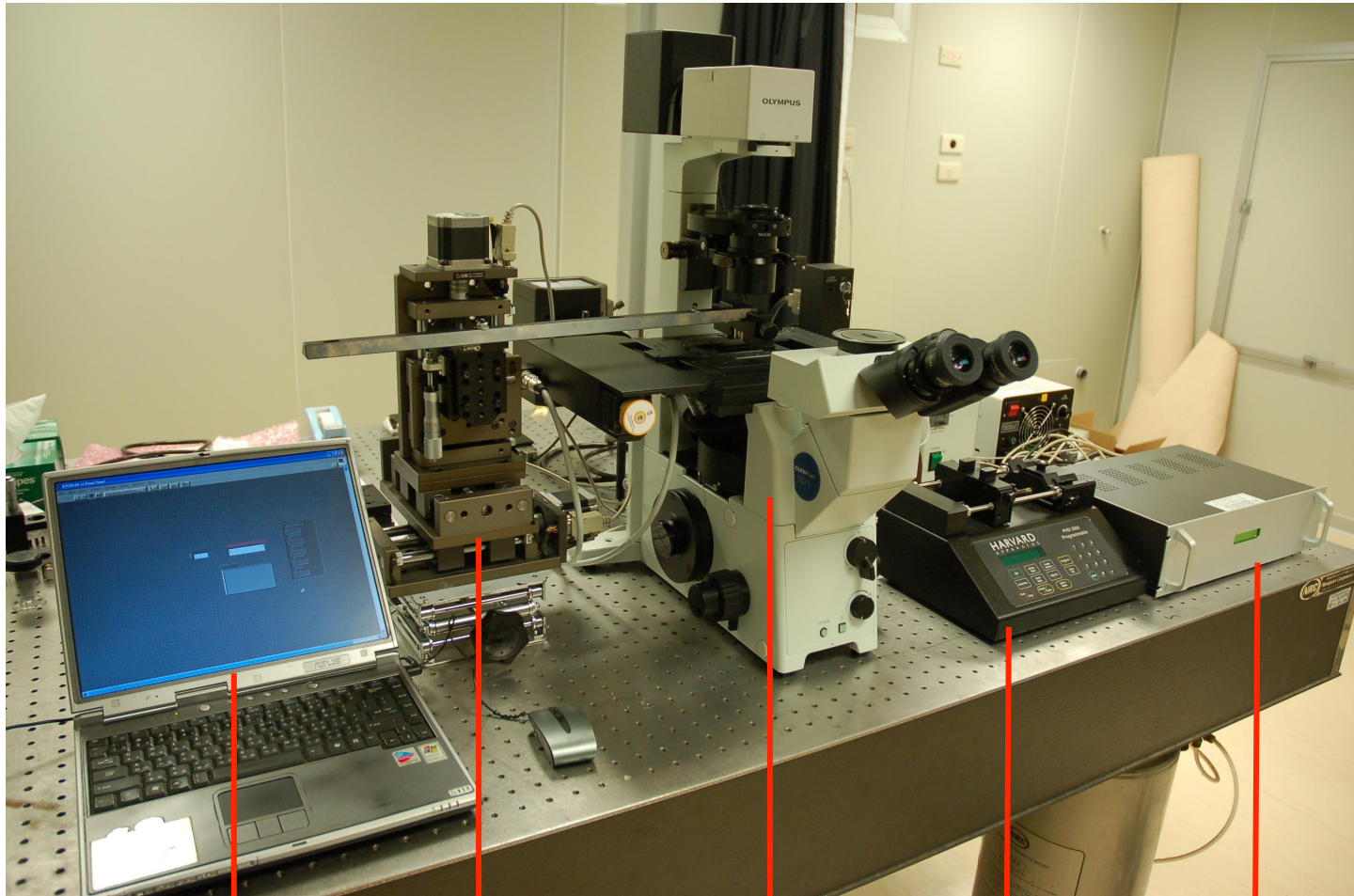
Force	Relative extension	RMS of fluctuation
10 fN	0.083	~ 815 nm
0.1 pN	0.5	~ 632 nm
1 pN	0.86	~ 262 nm
10 pN	0.97	~ 88 nm
100 pN	~2	~ 40 nm



Excellent low-force measurement technique



Experimental Setup



**LabView
Control
Panel**

**3-axis
Stage**

**Inverted
Microscope**

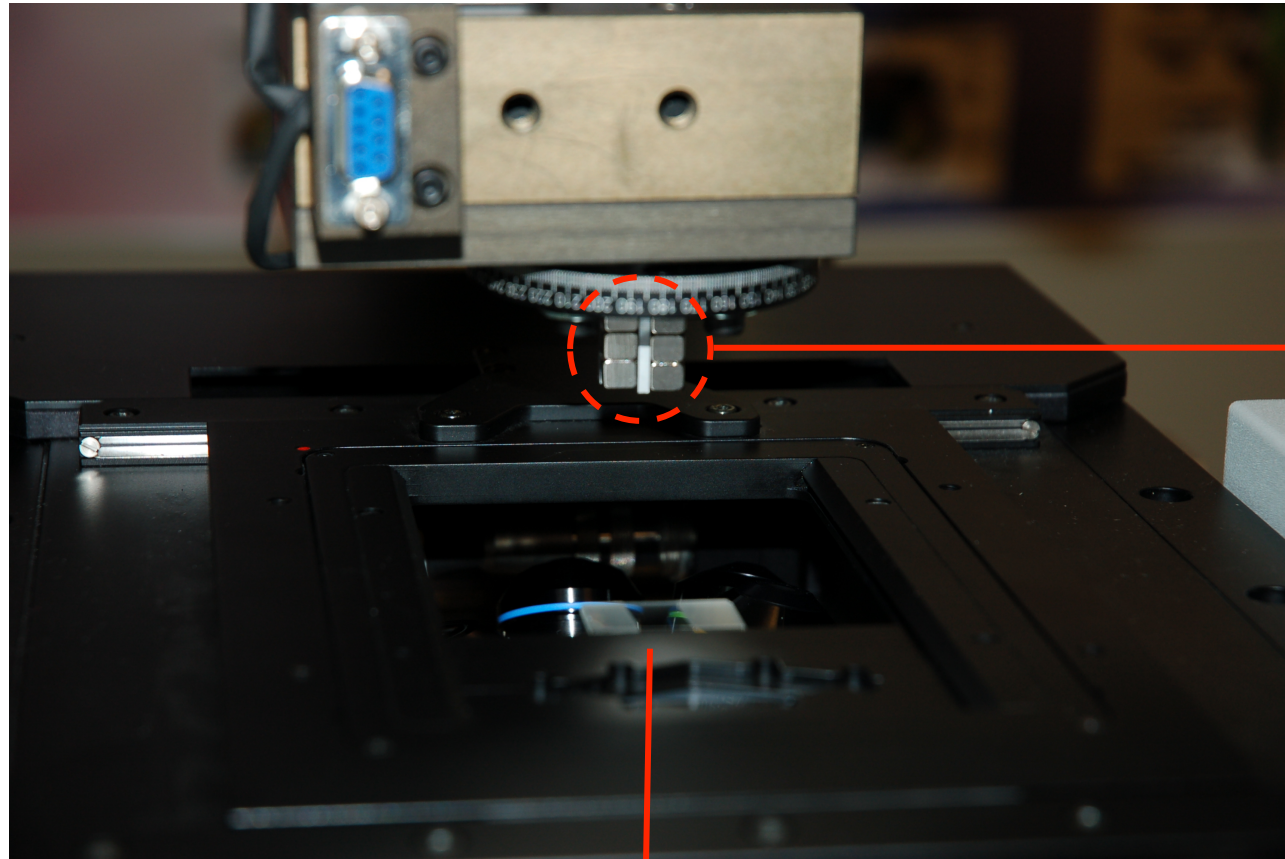
**Syringe
Pump**

Control Box

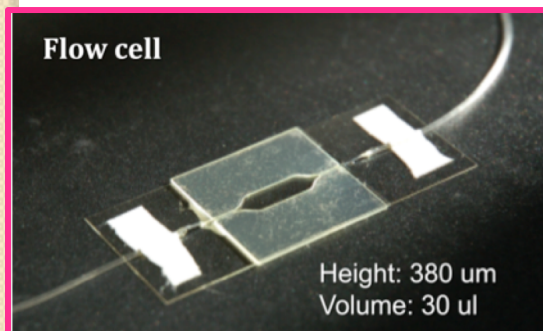


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Experimental Setup



**Permanent
Magnets**

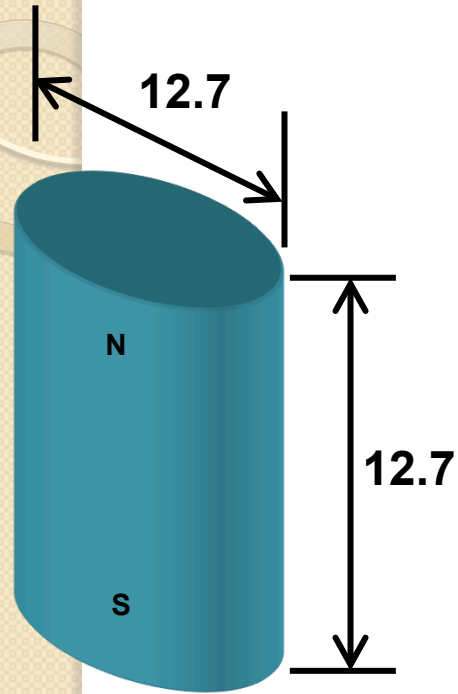


Microfluidic chip



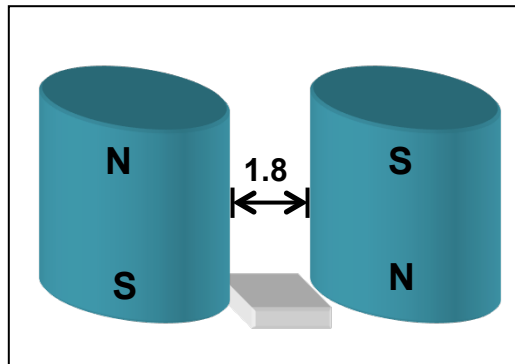
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Experimental Parameters



Permanent Magnet

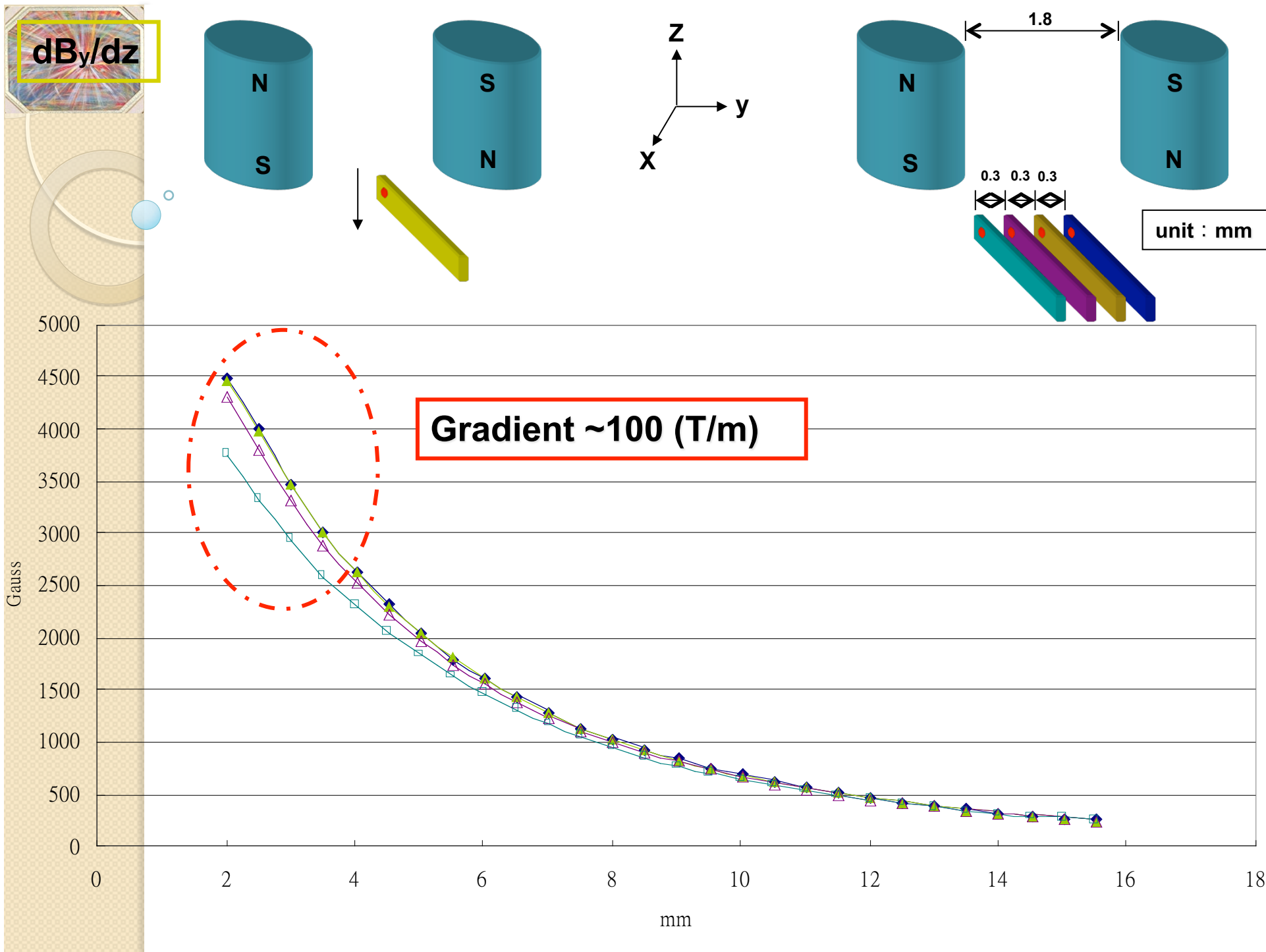
Permanent Magnet	Neodymium Iron Boron
Material	NdFeB
Diameter	12.7mm
Height	12.7mm
Gap	1.8mm
Maximum magnetic field	5490 Gauss

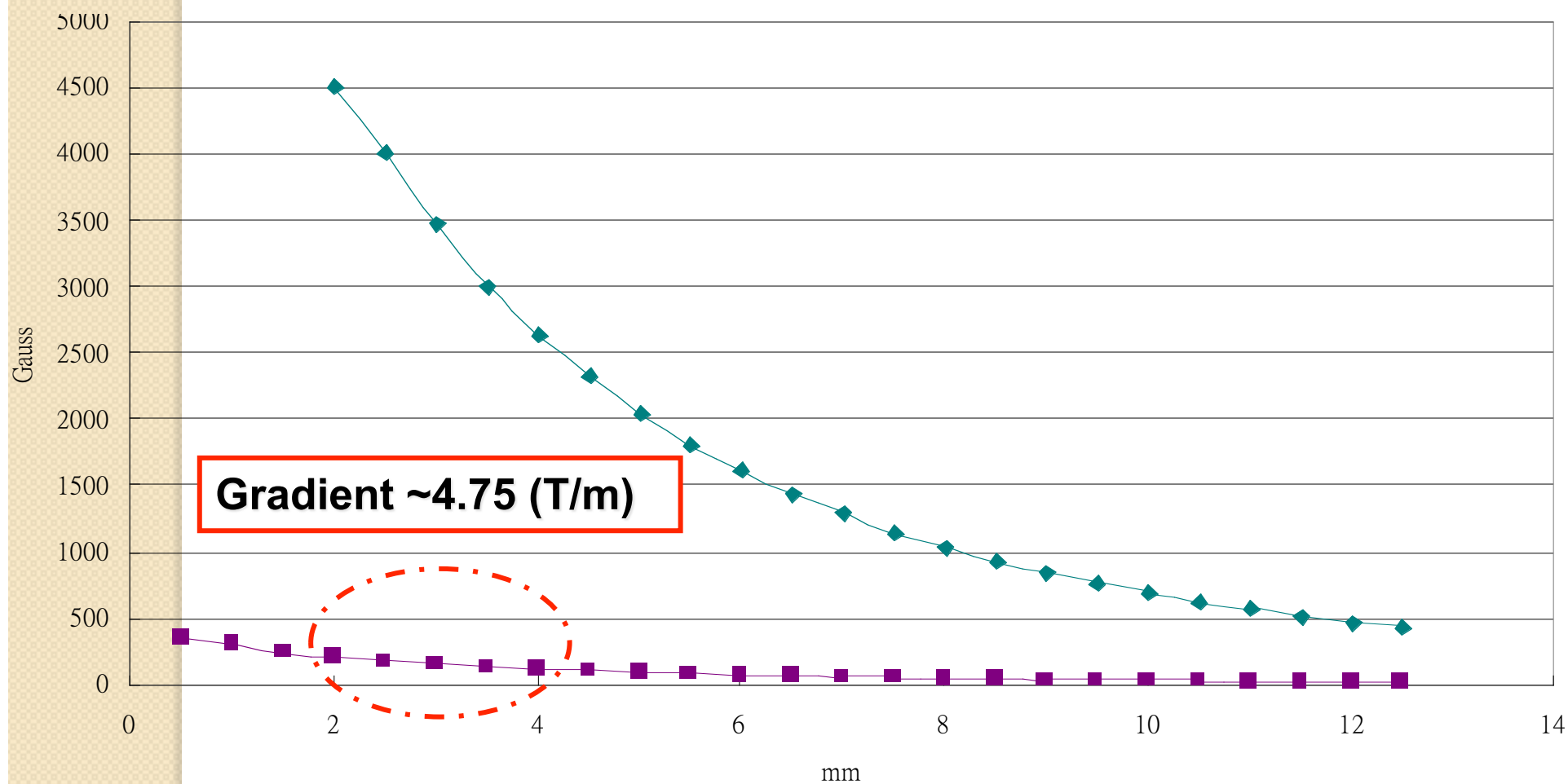
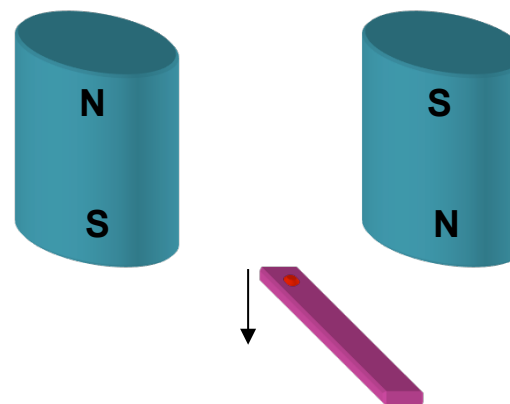
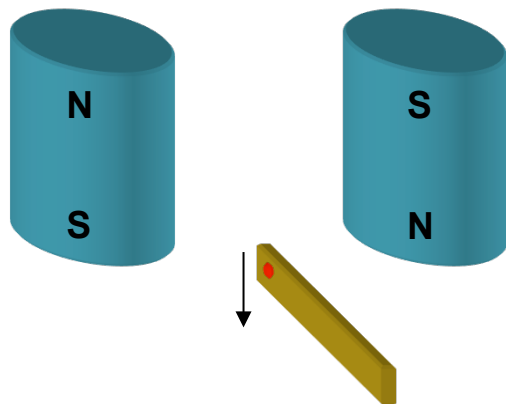
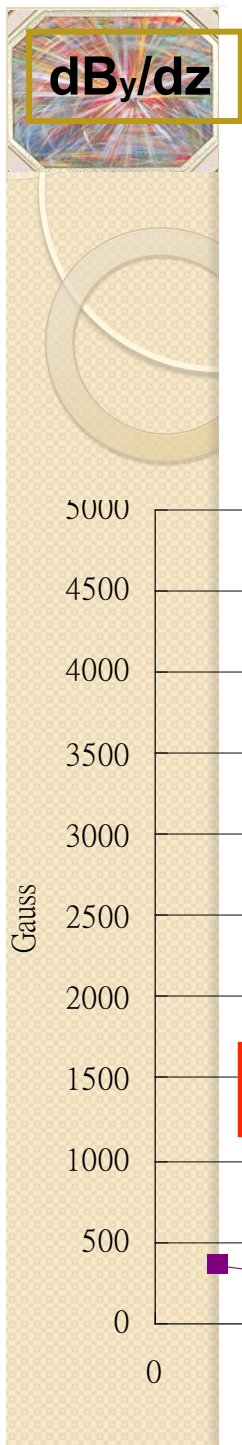


unit : mm

Gauss Meter

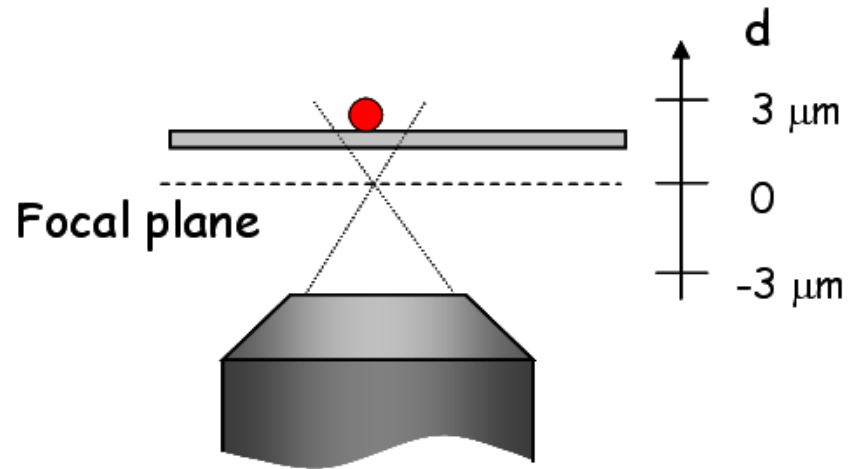




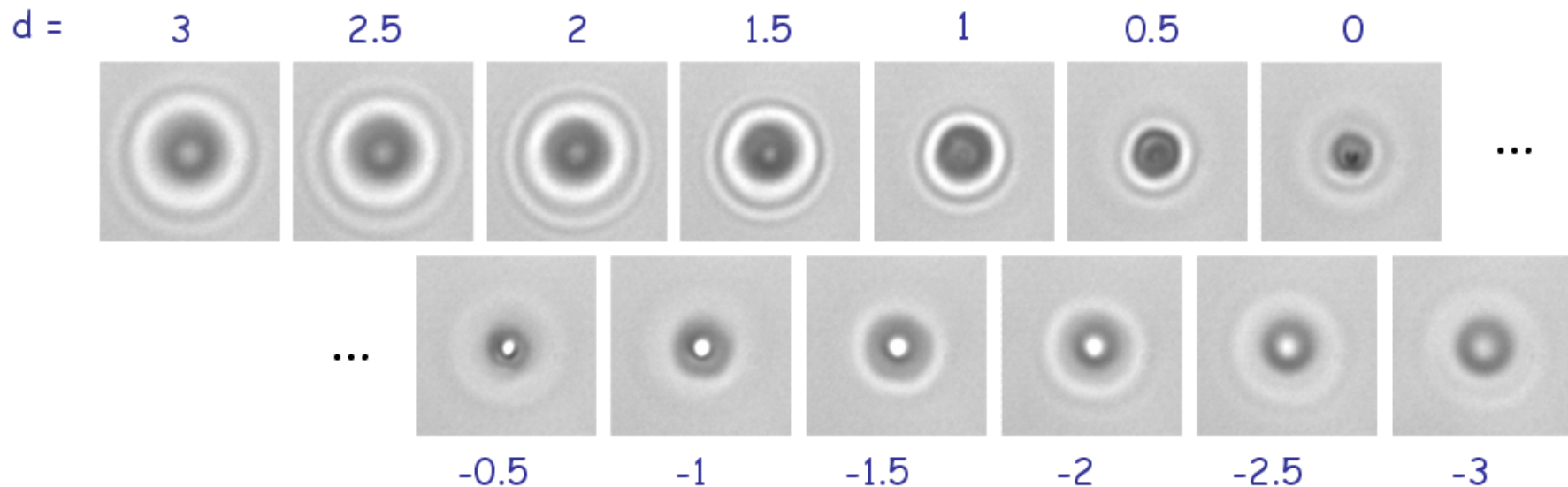




Single particle tracking system

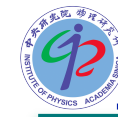
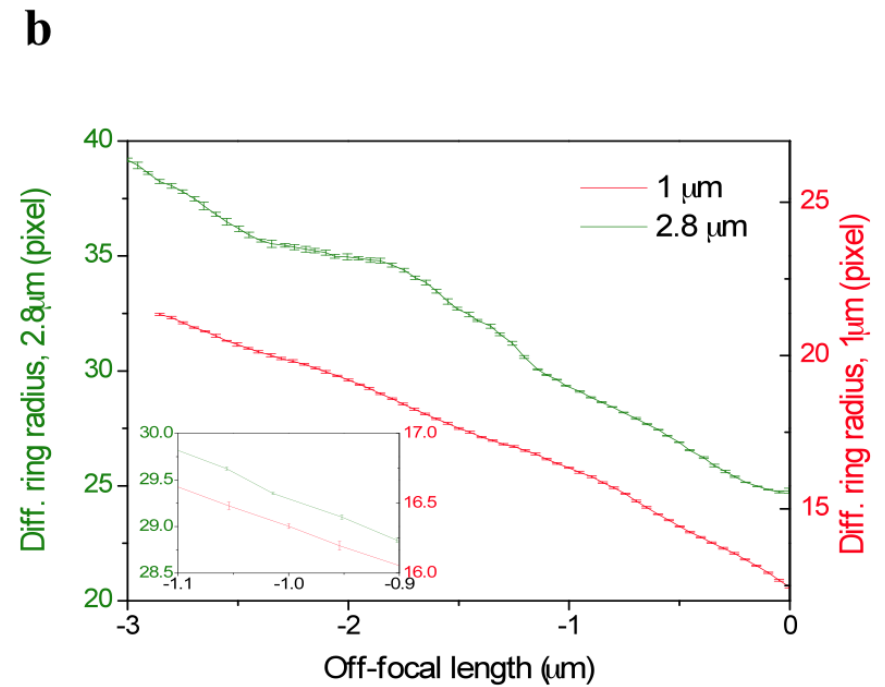
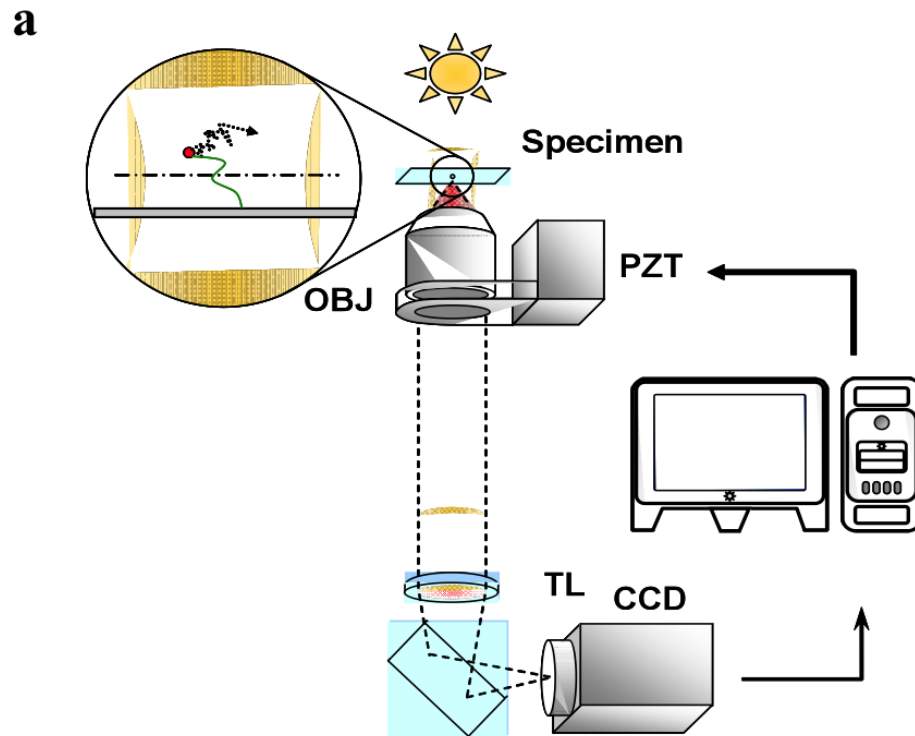


The diffraction rings determines the z position of the bead.

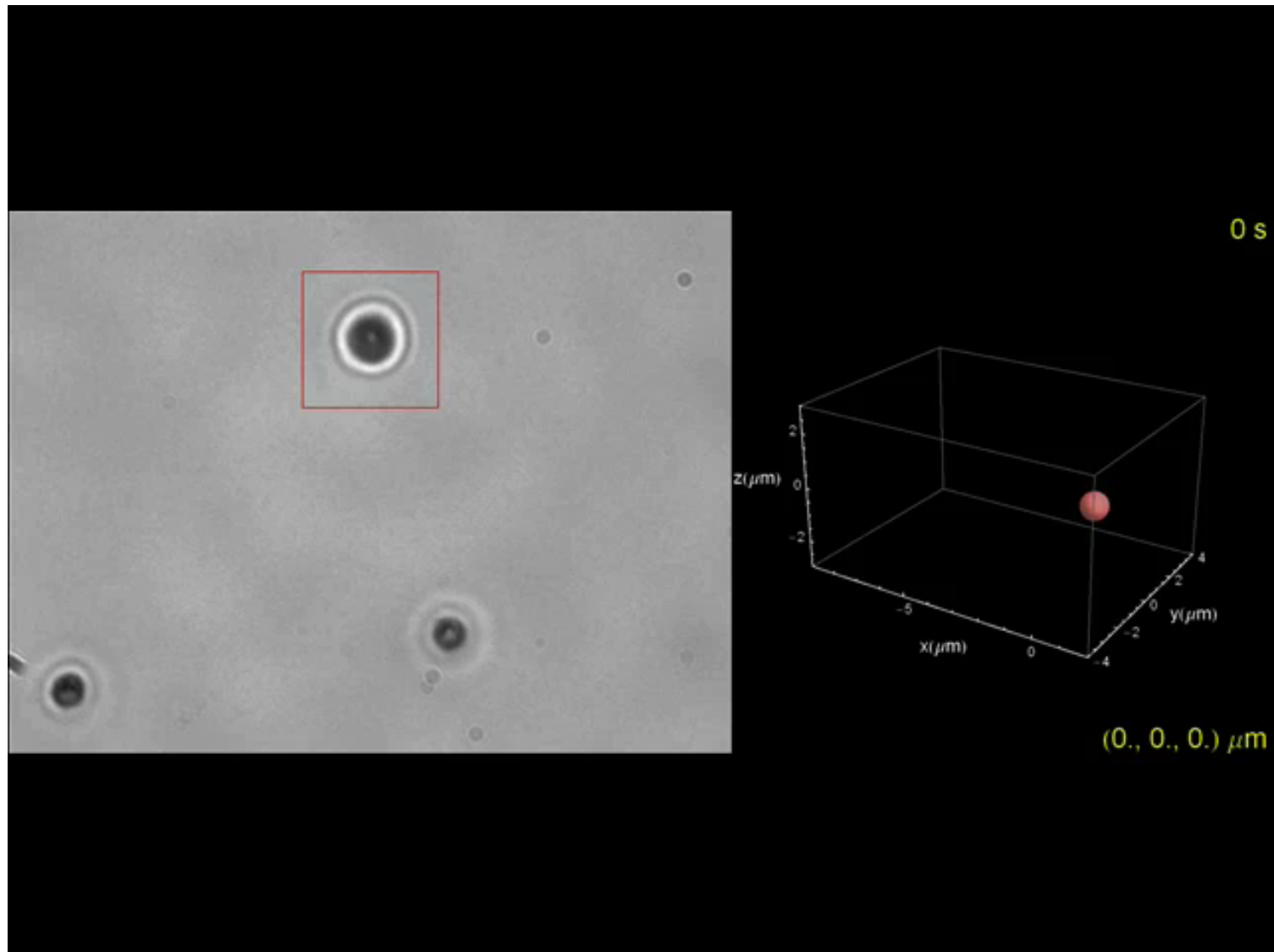


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Single particle tracking system — Scheme and calibration curve

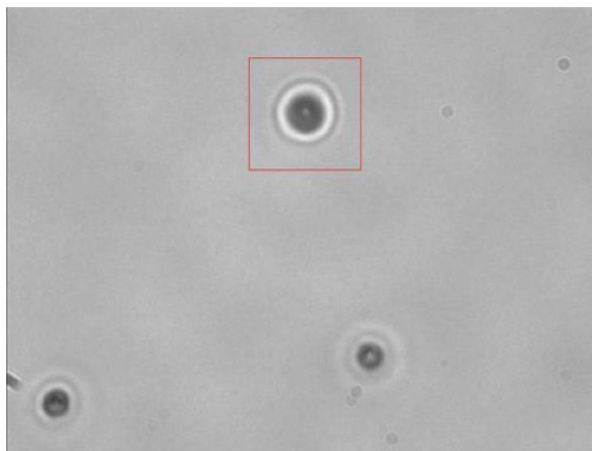


Free bead with tracking-I

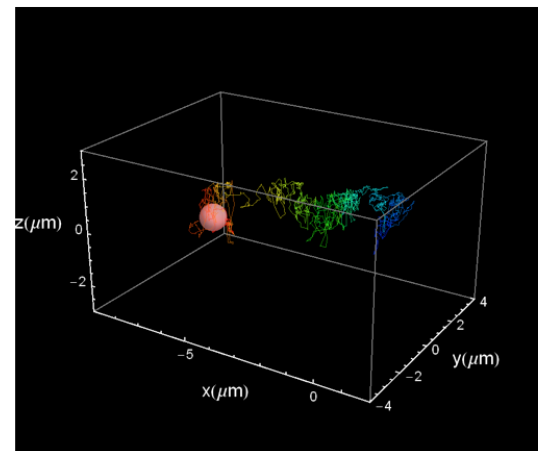


Free bead with tracking-II

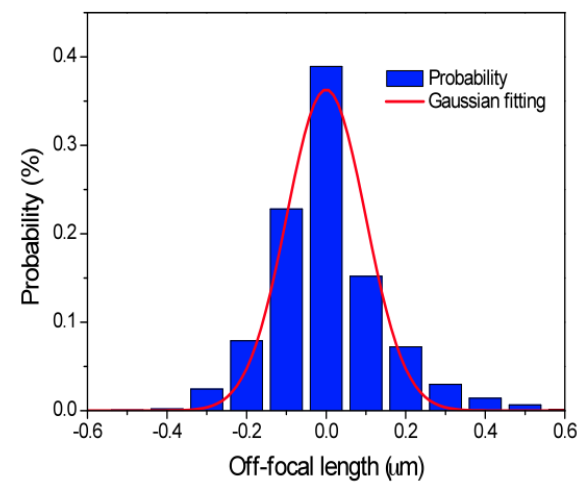
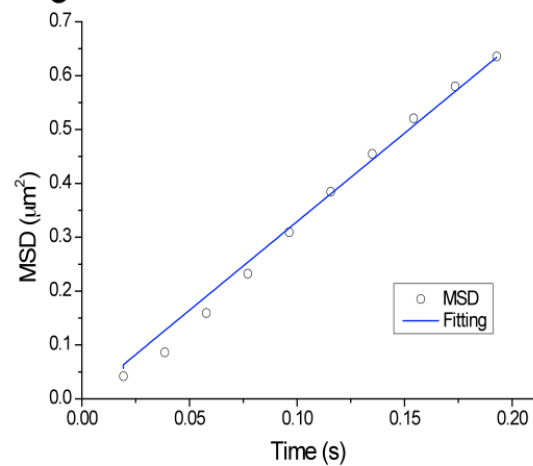
a



b

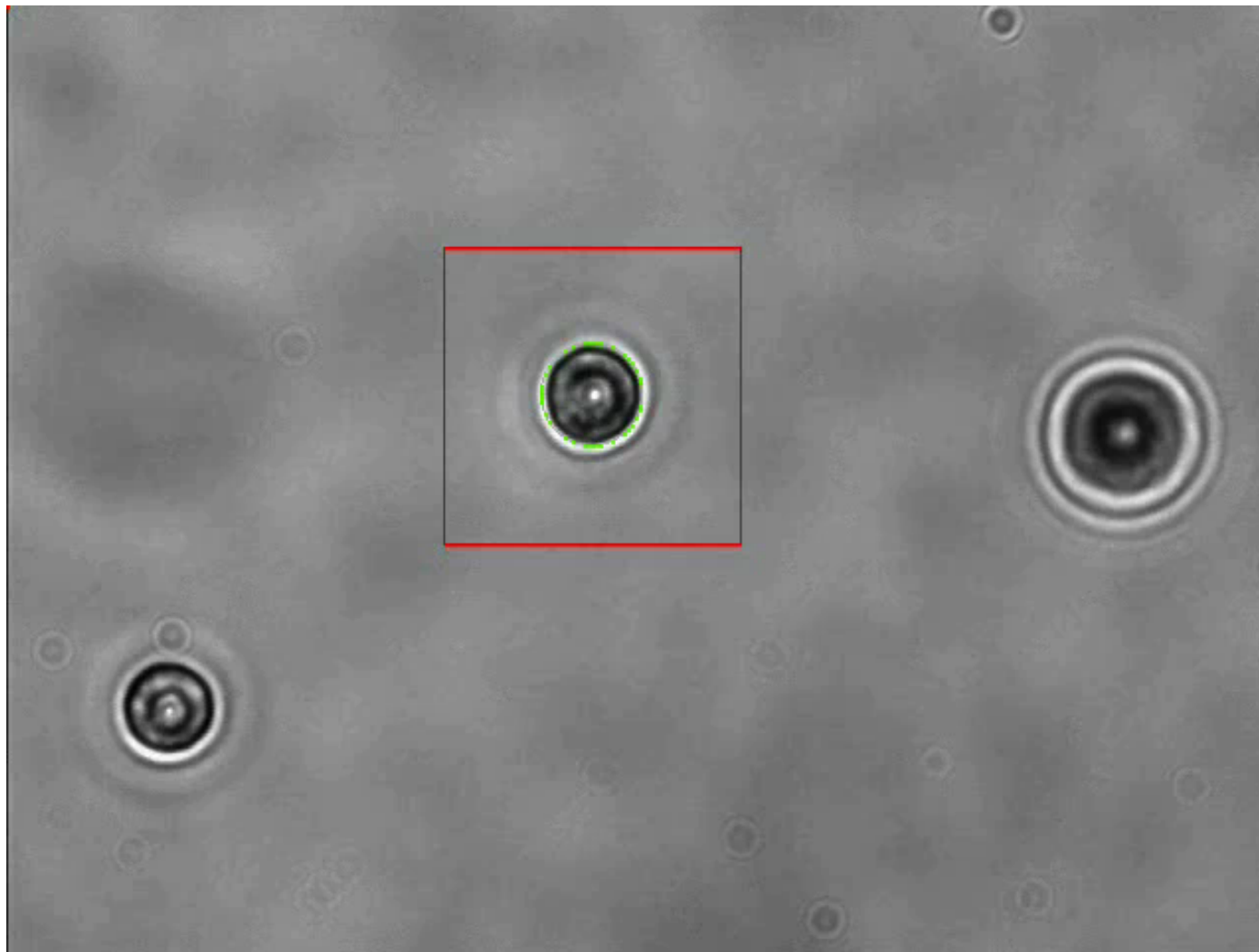


c

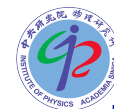
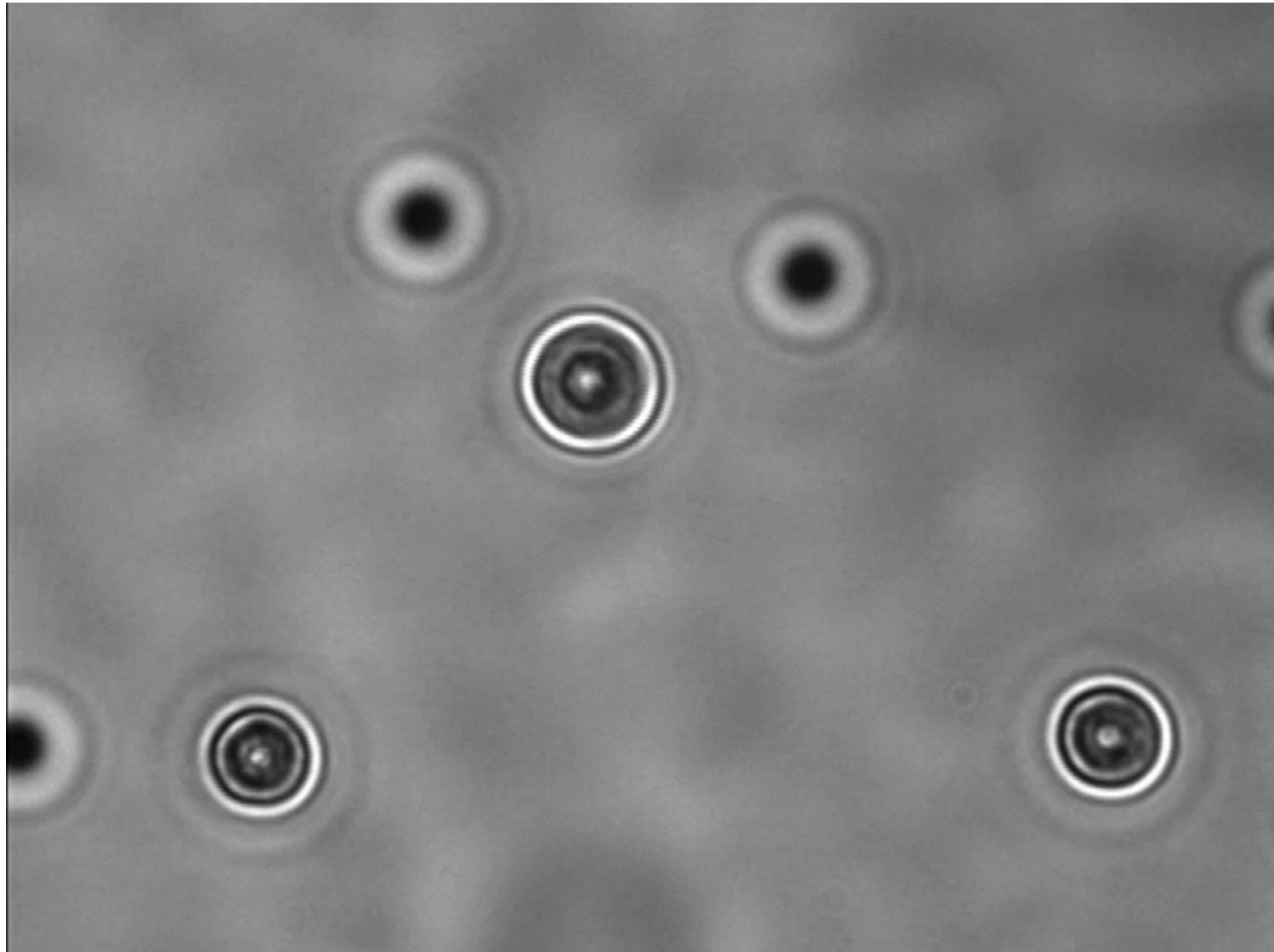


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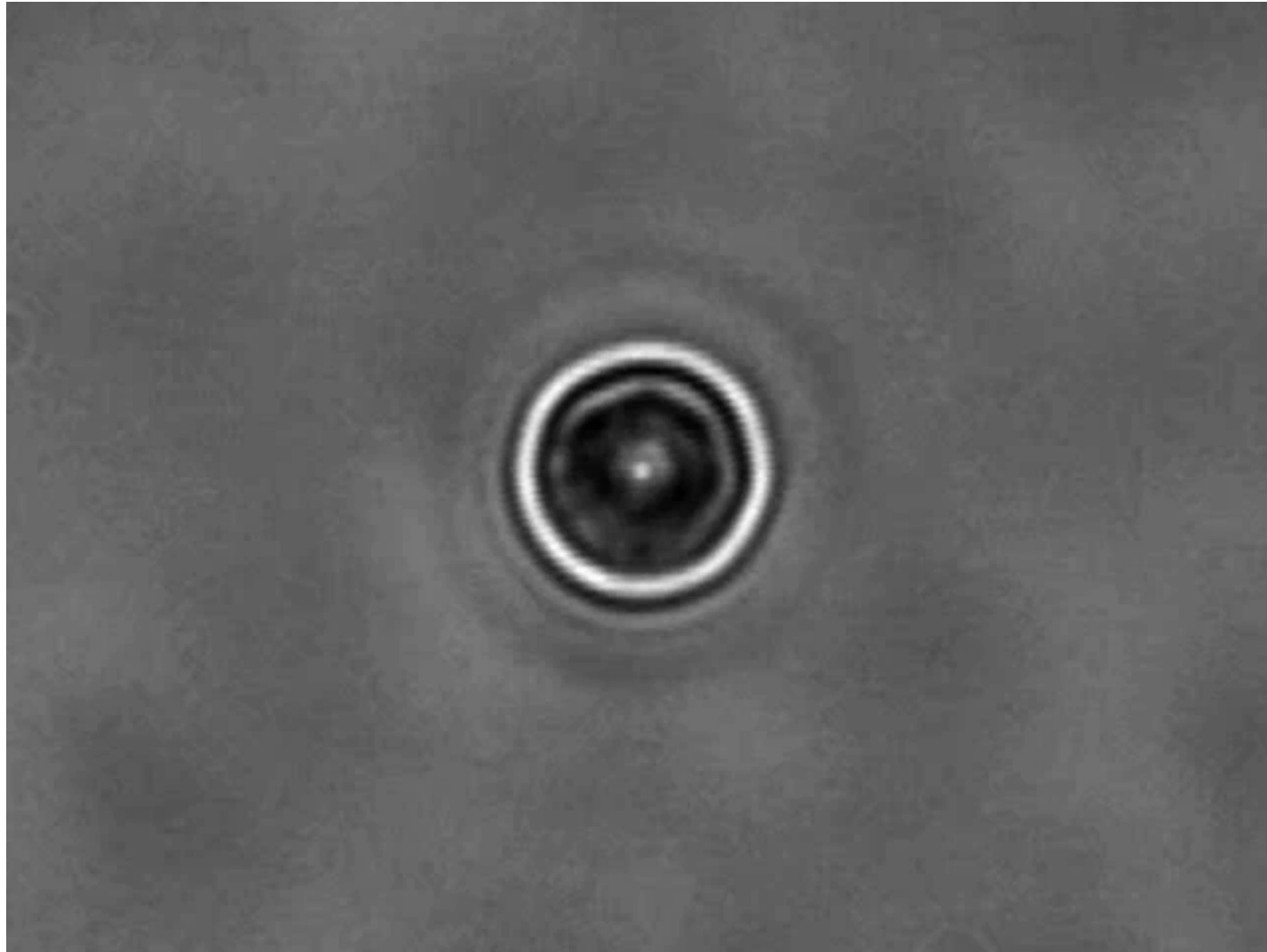
Bead-DNA tethering



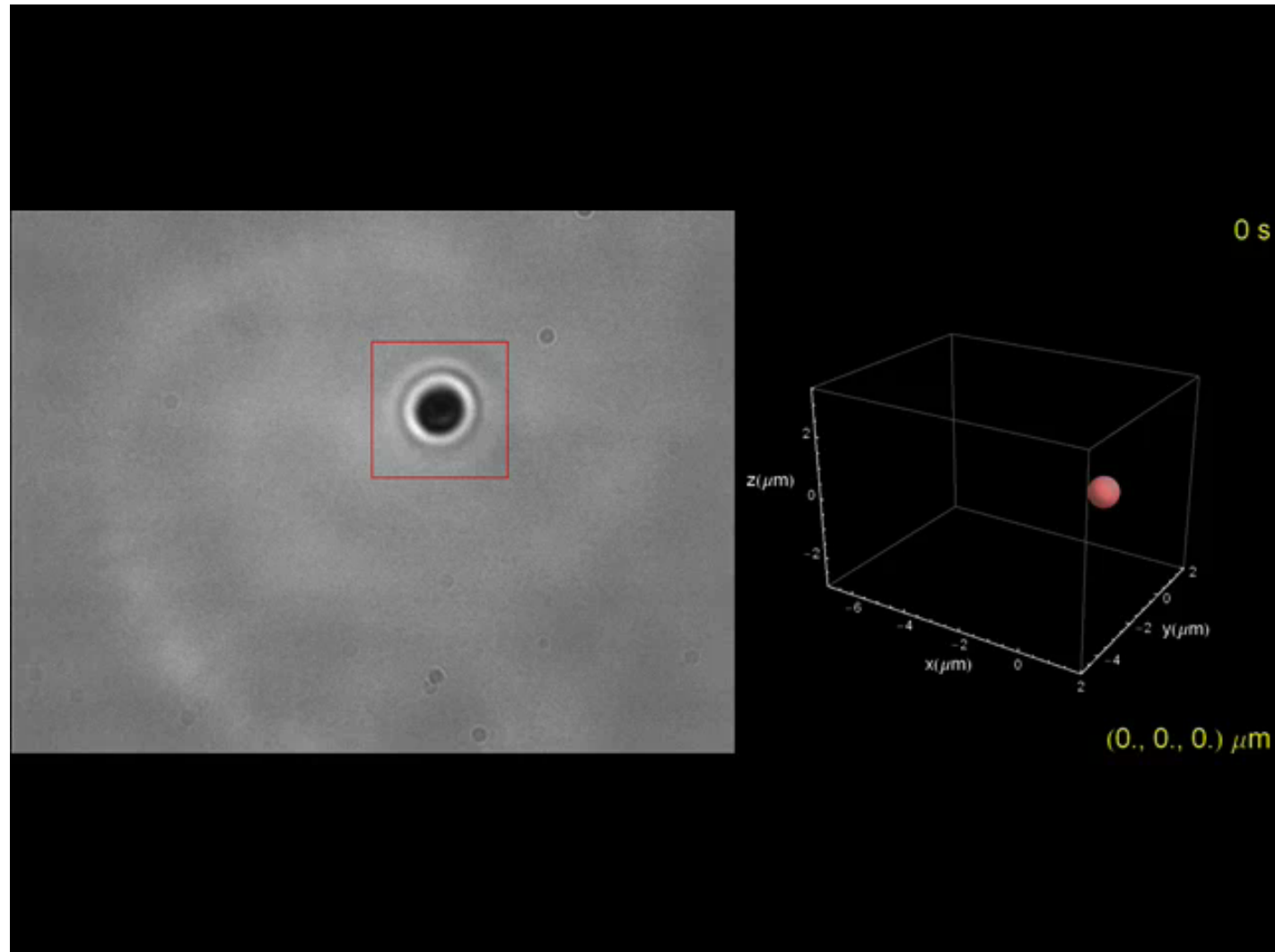
Multiple bead-DNA tethering



Bead-DNA rotation

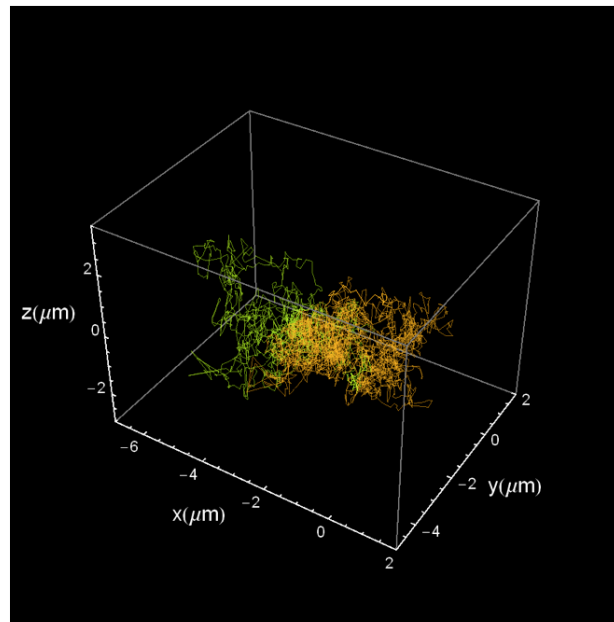


Bead-DNA tethering with drift-I

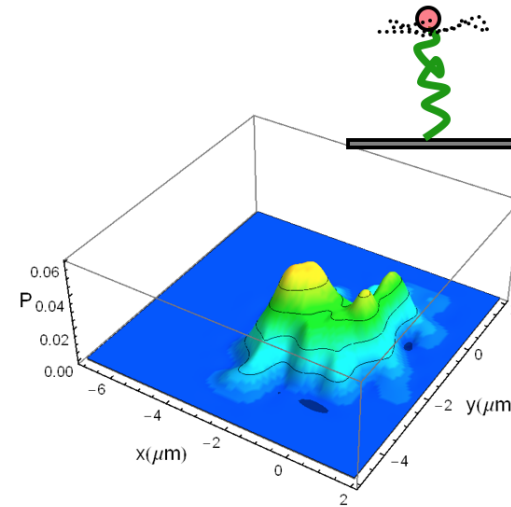


Bead-DNA tethering with drift-II

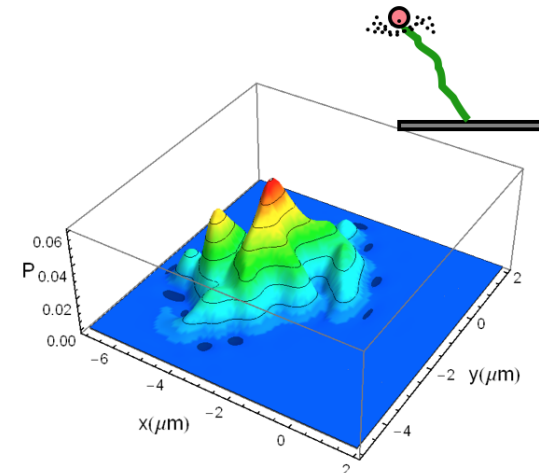
a



b

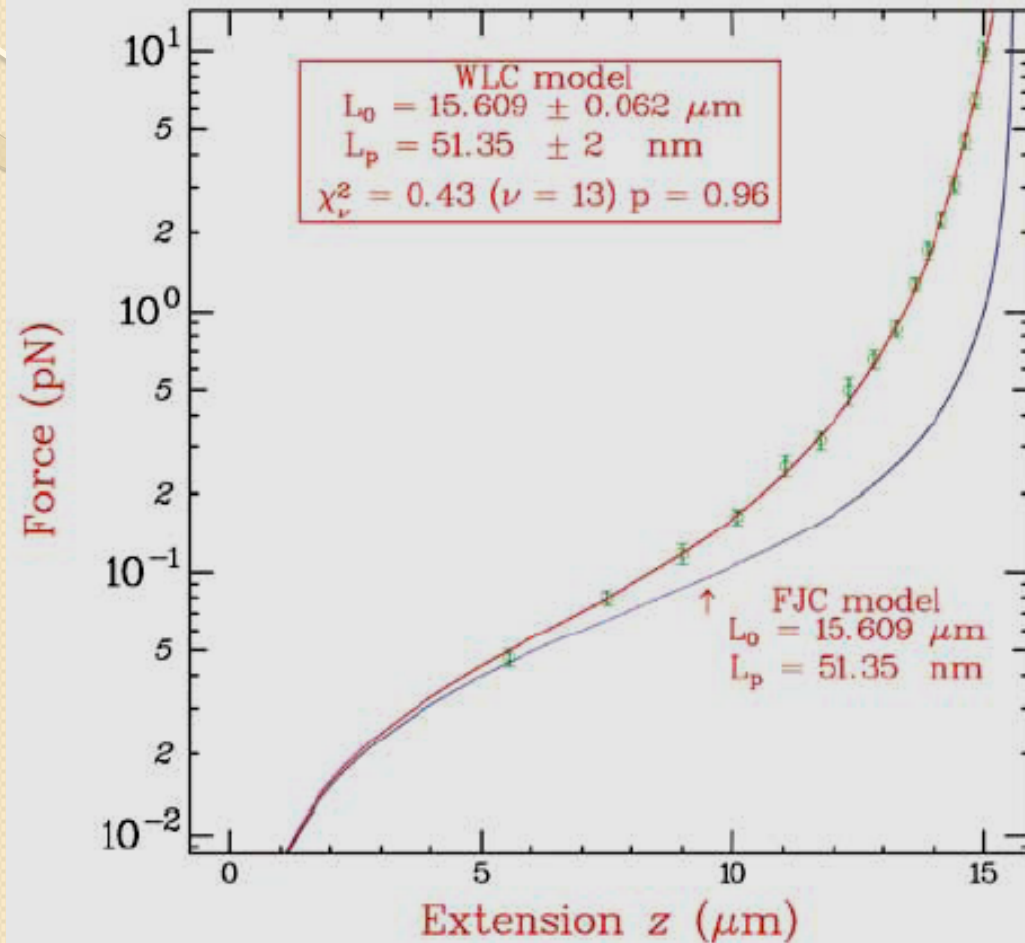


c



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Force-extension curve of λ -DNA



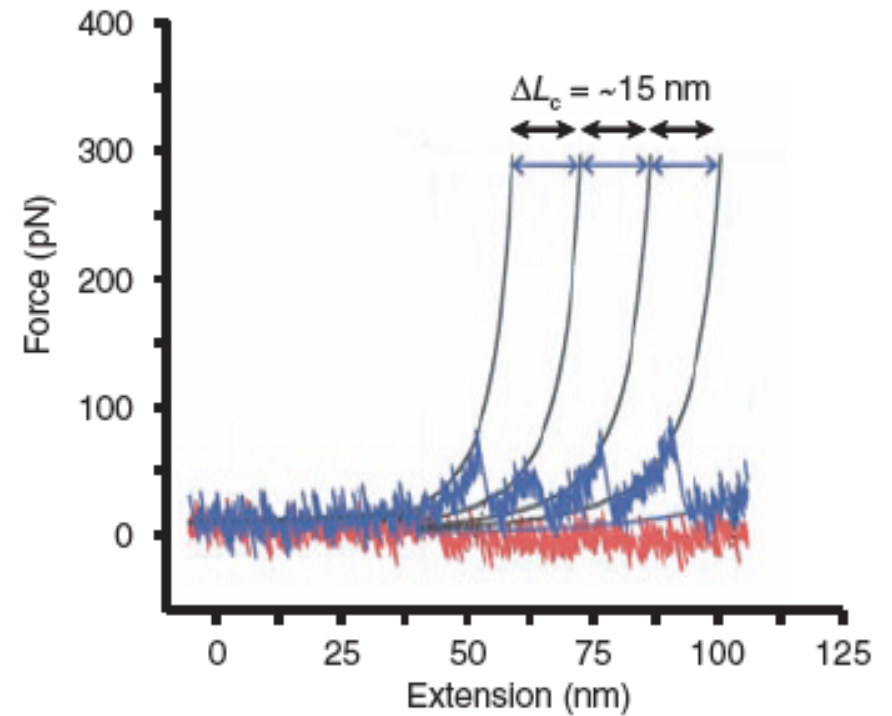
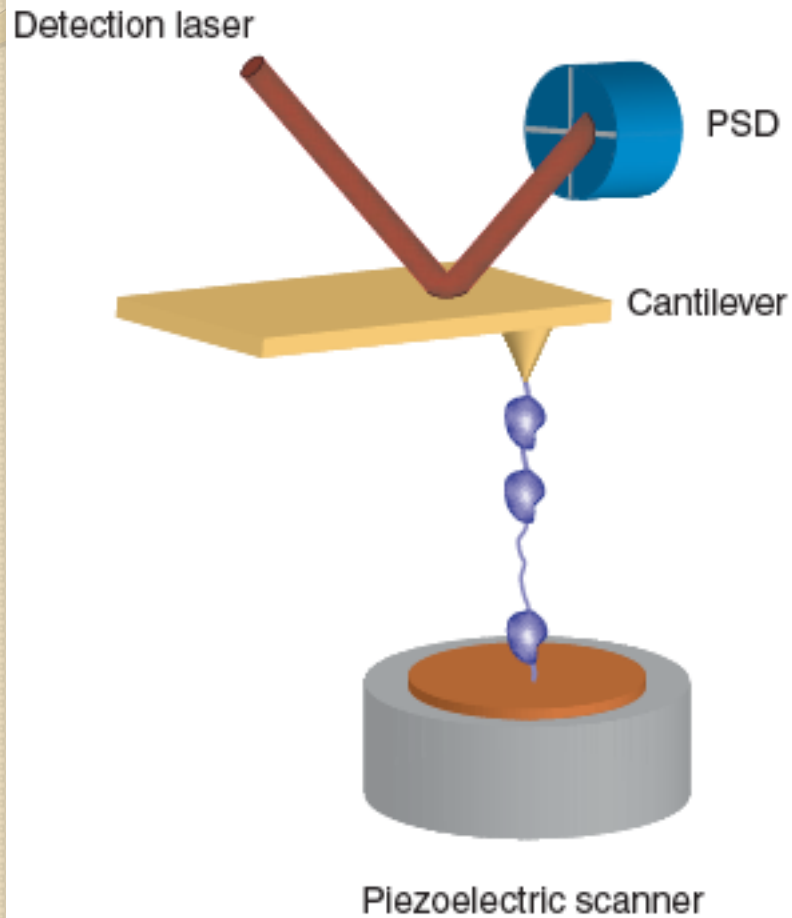
Worm-Like Chain Model:

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

A: persistence length

L: contour length

Atomic force microscopy



Protein elasticity: unfolding titin

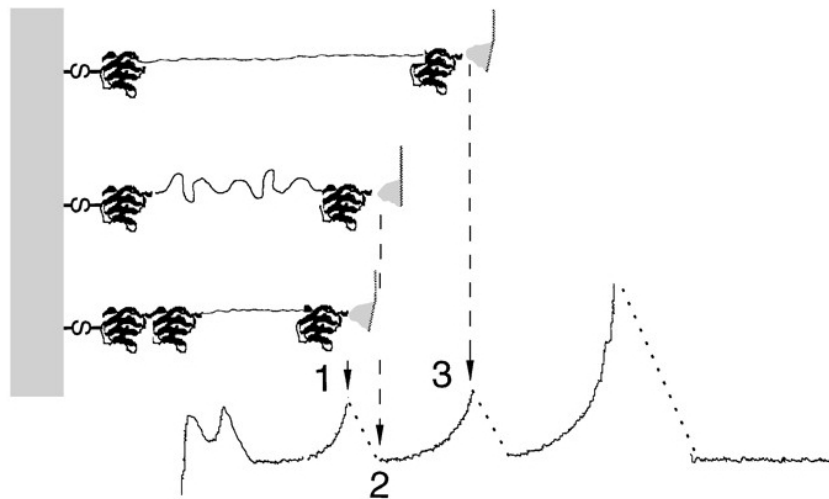
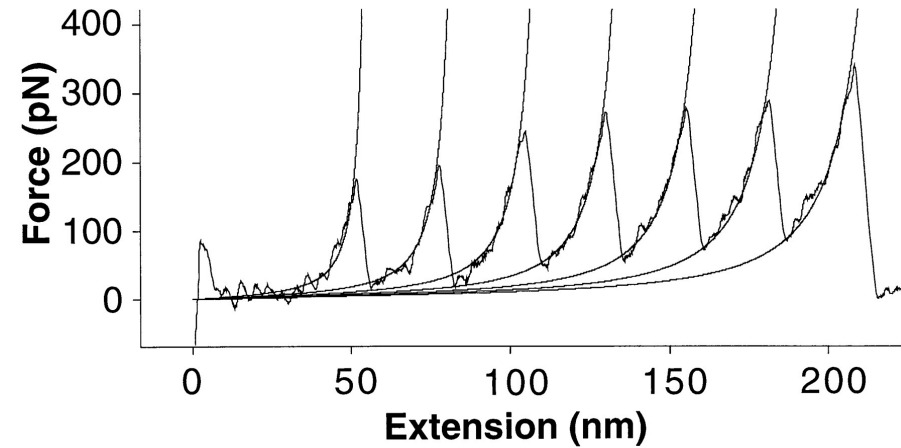
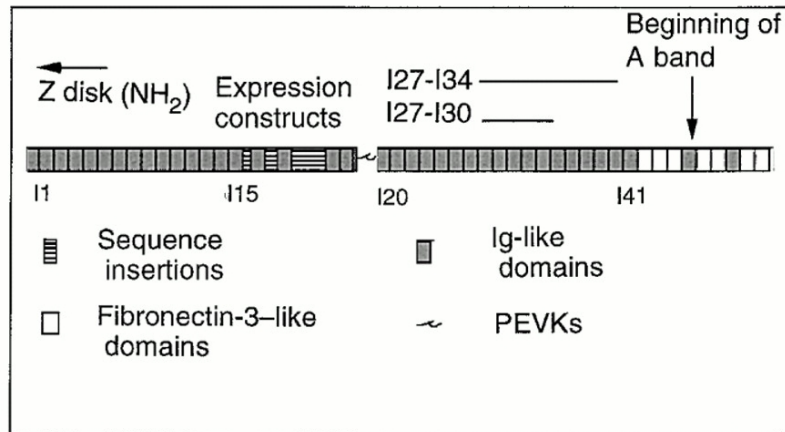


Table 1 | Comparison of single-molecule force spectroscopy techniques

	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



Further readings:

1. Block S. M. 1992. "Making light work with optical tweezers." *Nature* 360(6403): 493-5.
2. Svoboda K., Block S. M. 1994. "Biological applications of optical forces." *Annu Rev Biophys Biomol Struct* 23:247-85.
3. Neuman, K.C. & Block, S.M. Optical trapping. *Rev. Sci. Instrum.* 75, 2787–2809 (2004).
4. [http://www.stanford.edu/group/blocklab/Optical Tweezers Introduction.htm](http://www.stanford.edu/group/blocklab/Optical%20Tweezers%20Introduction.htm)
5. Strick, T., Allemand, J., Croquette, V. & Bensimon, D. Twisting and stretching single DNA molecules. *Prog. Biophys. Mol. Biol.* 74, 115–140 (2000).
6. Charlie Gosse and Vincent Croquette. Magnetic Tweezers: Micromanipulation and Force Measurement at the Molecular Level. *Biophysical J.* V82 June 2002 3314–3329.
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: from microns to nanometers and beyond. *Annu. Rev. Cell Dev. Biol.* 11, 241–265 (1995).